THE AMERICAN JOURNAL OF PHYSIOLOGY

EDITED FOR

THE AMERICAN PHYSIOLOGICAL SOCIETY

CONTENTS

(Continued on Cover 4)

Vol. LXXXIX—No. 2

Issued July 1, 1929

BALTIMORE, U. S. A.

1929

Entered as second-class matter, August 18, 1914, at the Post Office at Baltimore, Md., under the act of March 3, 1879. Acceptance for mailing at special rate of postage provided for in section 1103, Act of October 3, 1917. Authorised on July 5, 1918

Made in United States of America

THE AMERICAN JOURNAL OF PHYSIOLOGY

VOL. 89 JULY 1, 1929 No. 2

RESPONSES OF EXCISED INTESTINES TO ALTERATIONS OF ELECTROLYTE CONCENTRATIONS (Na, Ca, K)

R. W. WHITEHEAD[1]

From the Department of Pharmacology, School of Medicine, Western Reserve University, Cleveland, Ohio

Received for publication December 19, 1928

The proportions of inorganic cathions in the balanced salt solutions usually employed in studies on surviving tissues were originally determined almost entirely empirically or through imitation of the composition of blood serum or sea water (1). The analysis of the qualitative and quantitative rôle of each component cathion is still incomplete, and the reported results, especially those involving smooth muscle, have been somewhat confusing and contradictory. This is due in part to unjustified transfer of results obtained on one organ to other organs, but also to special difficulties inherent in smooth muscle. In this tissue spontaneous changes occur frequently and these may be mistaken for responses to experimental procedures. The experimental responses themselves vary considerably with the functional state of the tissue, depending on conditions which are in part not sufficiently known to be under control. The absence of a fixed "norm" renders it difficult to express the changes quantitatively; and the occurrence of qualitative changes in the type of contractions may render comparisons meaningless or misleading. These inherent difficulties may be partly overcome by increase of experience, partly by varying the experimental approach. Further study of the relationship of the ionic composition of the medium to functional activity of mammalian smooth muscle therefore seemed advisable. Intestinal muscle was selected for this purpose, partly because of its extensive use in investigations on smooth muscle, but also because two types of contractions, occurring under different conditions, may be studied in this tissue. The Magnus method was used for the sake of simplicity.

The particular object of the present experiments was the study of the functional changes resulting from the simultaneous alteration of the cath-

[1] Fellow in Medicine, National Research Council.

ions in the medium, whilst preserving their ratio to each other constant; and their comparison with changes in concentration of single component cathions, involving the ratio as well as the amount.

METHODS. Segments about 2 cm. long were taken from the ileum or jejunum of a freshly killed rabbit and suspended, as in the ordinary Magnus method, from a light muscle lever, in aerated, physiological solution kept at the temperature of 38 degrees Centigrade (±0.5 degree). A time signal, marking intervals of one-half minute, also served as a fixed abscissa for measuring changes in the length of the muscle.

The composition of the standard Locke was that of Sollmann and von Oettingen (2) for Magnus experiments, namely (per cent):

NaCl	0.9
KCl	0.042
$CaCl_2 \cdot 2H_2O$	0.024
$NaHCO_3$	0.008
Dextrose	0.1

The composition of this solution was changed according to a definite plan for all ionic alterations: the concentration of calcium, potassium and sodium was altered individually, involving a change of ratio of the particular ion to the other present; in addition, the concentration of calcium and potassium, and in another series of experiments, the concentration of calcium, sodium and potassium was altered simultaneously, involving no change in ratio, but only in the amount of ions.

Alterations in concentration were usually made by increments or decrements of one-fourth the amount of these ions in the standard Locke solution. In some experiments larger steps in concentration were taken (zero to normal, normal to 2½ times the normal, etc.). Descending concentrations involved the successive removal of one-fourth the amount of the ion or ions in question, down to complete absence; ascending concentrations were then made by the same steps to normal and in certain cases beyond the normal concentration (to twice or three times the content in Locke). Since segments may not recover quantitatively from the responses to hypo-standard concentrations, records were also taken on fresh segments beginning with the standard Locke solution and ascending by increments of one-half the standard concentration.

Descending concentrations were carried out by the addition of the requisite amount of a Locke solution lacking only in the ion or ions under investigation (e.g., in removing Ca and K simultaneously, a standard solution containing NaCl 0.9, $NaHCO_3$ 0.008 and dextrose 0.1 per cent, was added). The diluting solution was at the same temperature as that in the contraction chamber and was added from below, the original fluid being displaced and removed by overflow, thus avoiding exposure of the

muscle to the air. Wherever complete change of fluid to zero concentration of any ion was carried out, the fluid in the contraction chamber was replaced by washing with at least five times its volume of the new solution (free of one or several ions, as the case might be).

Ascending concentrations of ions were carried out by the addition of a Locke solution, in which the ion being investigated was present in concentrated form. For instance, for one-fourth the normal amount of calcium, a Locke containing 0.3 per cent of $CaCl_2 \cdot 2H_2O$ was added in amounts of 1.5 cc. to a contraction chamber which held 75 cc.; to make similar increments of K concentration, a solution containing 0.525 gram KCl per 100 cc. Locke was added in similar amounts.

Alteration of the concentration of ions would entail a decrease or increase of osmotic pressure, the possible effects of which it was thought best to control. A second series of experiments was therefore performed, paralleling the first in each case, in which increasing concentrations of either or both ions (Ca or K) was accompanied by a corresponding decrease in the concentration of sodium, to prevent increase of osmotic pressure. Experiments involving decrease of Ca and K were controlled by the addition of sucrose to maintain isotonicity. The effects produced by concentrations of calcium and potassium above normal (to 2 or 3 times the normal) were also checked by the addition of sodium chloride and sucrose, respectively, of a concentration equivalent to the osmotic pressure exerted by increments of Ca and K. To prevent osmotic changes when the sodium concentration was reduced, isotonic glucose solution was added.

The experiments in each series were repeated until it became possible to distinguish the typical response. The data were then averaged in terms of percentage of the initial function and the medians were plotted as charts, as in figure 1. The changes between one-fourth and zero concentration could not be charted, because they are largely qualitative.

Experimental results. *A. Simultaneous alteration of Ca and K concentration.* Simultaneous alteration of calcium and potassium was carried out first, to test the statement often made that the quantity of ions present is practically unimportant if their ratio is preserved. The results of reducing calcium and potassium concentrations simultaneously by decrements of one-fourth the normal amounts in Locke are shown graphically in figure 1 (Ca + K).

Alterations to one-fourth the normal amount of both ions was accompanied only by quantitative changes in functions. The results were particularly uniform as regards the rate of the pendular rhythm, which decreased progressively as the solution became poorer in potassium and calcium. The amplitude rose smoothly and progressively with successive dilutions from 1 to $\frac{1}{4}$ concentration, while the diastolic tone fell with the rate ("tone" is used as synonymous with "tonus" and in this paper, the

"diastolic tonus" is meant when only tone is stated; namely, the state of sustained tension remaining at the completion of an active relaxation. Systolic tone, or tonus, is the tension at the height of an active contraction).

In contrast to the relatively slight changes in functions which appear with mere reduction of Ca and K concentration from normal very decided alterations occur, qualitatively as well as quantitatively on passing to

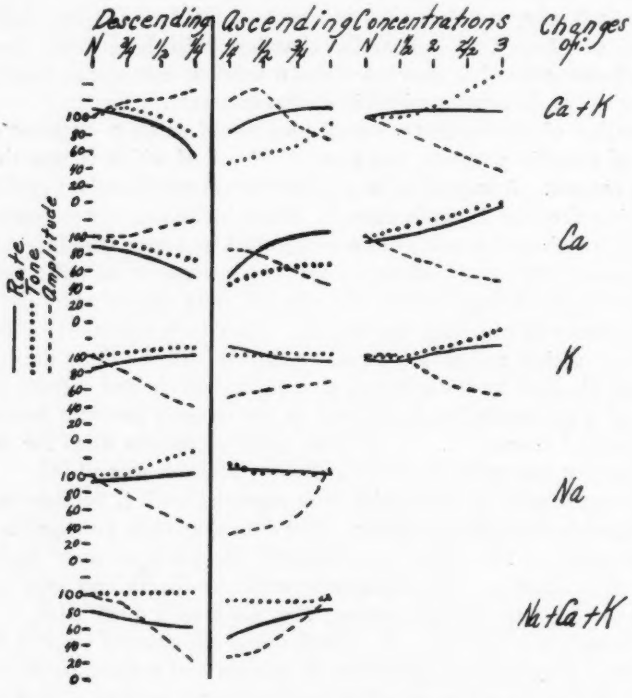


Fig. 1. Functional changes with alterations of cathions in Locke's solution (Magnus intestine). Abscissa, fractions of normal concentration; ordinate, amplitude, rate and tone. Rate in absolute units; amplitude and tone in arbitrary units.

zero concentration of these ions, either directly from normal or from one-fourth the normal concentration (fig. 2). Qualitatively, there is a change from the pendular type present at one-fourth normal concentration of Ca and K, to a periodic type of rhythm. This is due to the superposition of incomplete contraction of successive levels of the segment, instead of single contractions of the whole segment (3). This division of contractions results in dissimilar amplitude, and in quickened rate if the partial contractions, i.e., the humps on the curves are counted. The diastolic tone

rises, but if the change is abrupt, this may be preceded by a temporary fall. The amplitude continues to decrease progressively with the sojourn at zero concentration of these ions, until gradually the spontaneous contractions die out, usually in about half an hour. The tone does not remain elevated but tends to fall gradually below the original level. The results were not materially different when sucrose was present to make up the deficit in the osmotic pressure occasioned by the removal of calcium and potassium.

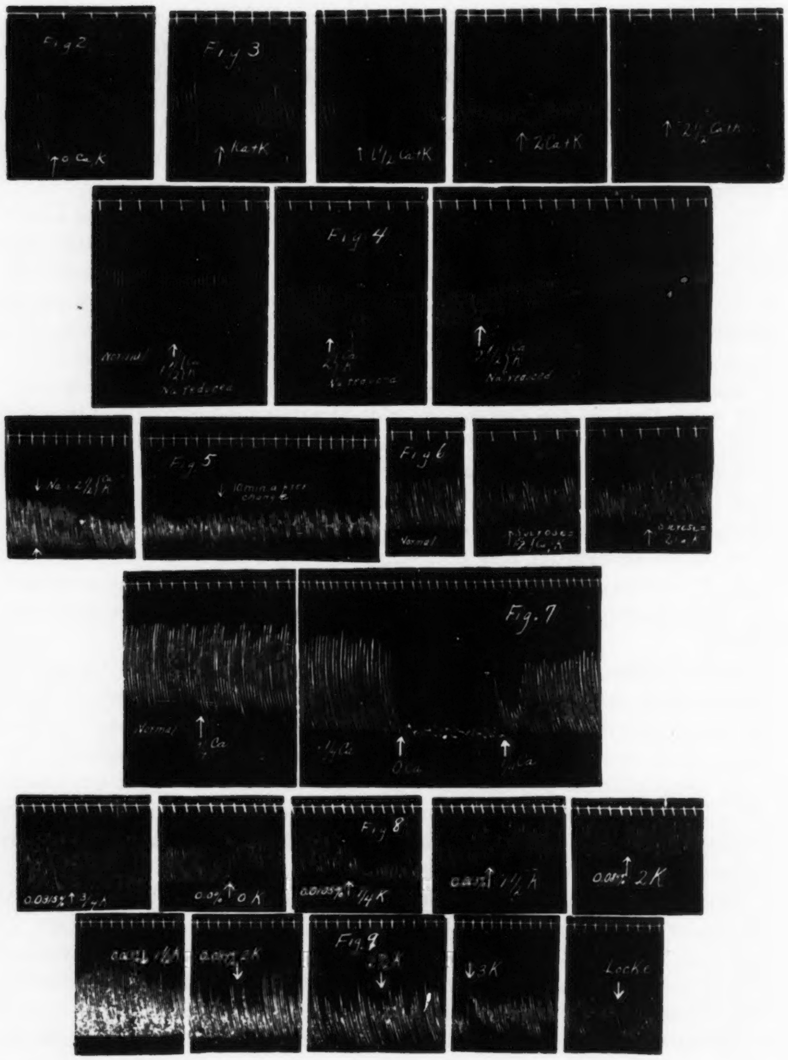
In order to determine whether the absence of Ca and K from the Locke solution permanently injures or destroys the viability of intestinal segments, these were kept in a solution containing NaCl, $NaHCO_3$, and dextrose for periods varying from five minutes to four hours, maintaining constant aeration and temperature. At the end of the allotted time the segments were returned to normal Locke and a tracing was taken as the tissue resumed activity. This occurred even if the intestinal segments had been kept in the sodium chloride-bicarbonate-dextrose solution for four hours. The return of functional activity was generally quite good but not quantitative, especially if the exposure had been prolonged; the rate was increased to faster than normal while the amplitude was decreased; the tone tended to rise with the sojourn in Locke.

Addition of calcium and potassium by increments of one-fourth the normal were next made, from zero to $\frac{1}{4}$, $\frac{1}{2}$, $\frac{3}{4}$ and normal concentration and in some experiments to above the amount in Locke (to three times Ca and K).

Segments that had been transferred to a solution free from Ca and K and to which these cathions were added in graded fractions showed definite functional alterations. Even one-sixteenth of the standard concentration produced a marked change of the periodic type toward the normal; with three-sixteenths the contractions were almost regular and with one-fourth they were quite normal. Accompanying the qualitative change the rate became slower, often one-half the normal, while the diastolic tonus was reduced and the amplitude was increased.

The functional changes produced by addition of Ca and K above one-fourth to one-half, three-fourths and normal and beyond this to concentrations of $1\frac{1}{2}$ to 2 times those in Locke are chiefly quantitative and except for some lagging are the reciprocal of those resulting from reduction of these cathions by the same amounts (fig. 1, Ca + K).

Increase of calcium and potassium concentration above normal results in a smooth continuation of the effects noted on going from one-fourth to normal concentration of Ca and K, namely, progressive increase of tone and decrease of amplitude. The rate does not increase beyond the maximum attained at three-fourths to normal concentration. In many experiments periodic waves appear when the concentration of these ions reaches $1\frac{1}{2}$ to 2



Figs. 2–9. Tracings of rabbit's intestine by Magnus arrangement. The upperline is the time in half-minutes. Changes of the concentration of the ions are indicated in terms of their concentrations in the ordinary Locke's solution.

times the normal (fig. 3). It was considered possible that the onset of these periodic contractions might be due to the increase of osmotic pressure incident to the addition of Ca and K. This was tested by preventing the rise of osmotic pressure, decreasing the sodium chloride in proportion to the molecular ratio of the additional $CaCl_2$ and KCl. When this was done, contractions usually remained pendular with ascending concentrations of Ca and K above normal (fig. 4) whilst the tone increased, and the amplitude decreased similar to that occurring in the uncompensated series. In order further to check the possibility that the incidence of peristaltic waves might be osmotic in origin, two other control series of experiments were performed; sodium chloride and sucrose were added respectively in concentrations which would exert the same osmotic pressure as the increments of Ca and K. For instance, increase of NaCl from 0.9 per cent to 1.0071 per cent or the addition of sucrose (1.165 per cent) was taken as osmotically equivalent to the addition of $CaCl_2$ and KCl to two and a half times the normal. These conditions also resulted in periodic contractions (figs. 5 and 6). We feel justified, therefore, in view of these results, in assuming that the periodic waves which appear at a concentration of $1\frac{1}{2}$ to $2\frac{1}{2}$ Ca plus K are due, largely at least, to the increase of osmotic pressure entailed by such increments.

B. Functional responses to alterations confined to the Ca ion. Since simultaneous alteration in calcium and potassium content of Locke solution produced definite changes in functions, even though the ratio was practically constant, the part that each of these plays in the result remained to be determined.

The alterations of calcium concentration were found to give the more important changes in functions and the results upon analysis were found to be closely allied to those following the simultaneous alteration of both Ca and K.

Starting with segments of intestine in Locke, reduction of Ca concentration by decrements of one-fourth normal concentration, from one to one-fourth, produces the effects shown graphically in figure 1 (Ca). The alterations in physiologic activity over this range of concentration are chiefly quantitative. The most characteristic change is again in the rate of contraction which is progressively diminished. The amplitude tends to increase, while the diastolic tonus decreases.

Removal of Ca directly from normal or from one-fourth the normal amount of Ca results in qualitative as well as quantitative changes (fig. 7). The pendular contractions become incomplete, leading to periodicity of rhythm, quite as with simultaneous deprivation of Ca and K. The rate and amplitude also behave similarly. The tonus, however, shows a significant difference: for it rises when both Ca and K are removed; whereas it remains unchanged or falls if Ca alone is withdrawn. The survival of

contractions constitutes another significant difference. When Ca and K are both withdrawn, the contractions become progressively weaker and are usually arrested within half an hour. When Ca alone is withdrawn the contractions and the tone tend rather to recover with time. Actual arrest does not occur within two hours.

Addition of one-fourth the normal amount of calcium to the calcium-free solution results in an almost immediate return to the pendular type of rhythm, accompanied by rise of tonus (fig. 7).

Responses following increments of Ca from one-fourth to normal consist chiefly in quantitative functional changes reciprocal to those accompanying reduction of this cathion, namely, progressive increase in rate and a smooth increase in tone, with corresponding decrease of the amplitude.

The increase of rate and tone, and the decrease of amplitude becomes progressively greater as the concentration of calcium rises above normal (fig. 1, Ca). Periodic tonus waves appear and increase progressively, essentially as with simultaneous increase of Ca and K. The periodicity, however, may occur even if the osmotic pressure is compensated by reduction of sodium chloride; whilst it does not appear with increments of both ions when NaCl is reduced.

As has been shown by Sollmann and von Oettingen (3), buffering with sodium phosphate instead of bicarbonate acts as reduction of the concentration of calcium. In accordance with this the present experiments also showed that when calcium was reduced in solutions buffered with 0.021 per cent sodium phosphate (crystals) the periodicity characteristic of calcium deprivation began to appear earlier, namely, often with half the normal concentration, that the effects of adding calcium were less marked than with equivalent concentrations buffered with 0.008 per cent sodium bicarbonate.

C. Functional responses to alterations confined to the K ion. Although the phenomena resulting from Ca alterations are very similar to those which occur when Ca and K are changed simultaneously, this does not mean that potassium effects are insignificant. In fact, this ion produces quite marked changes, in general, the reverse to calcium; but the latter (Ca) is so much more powerful that in its presence the potassium effects show themselves only imperfectly, except in respect to tone.

The changes on reduction are chiefly quantitative from the normal percentage to one-fourth the normal; the rate and tone tend to increase slightly while the amplitude decreases progressively and markedly (fig. 1, K, and fig. 8).

The prompt qualitative change to periodicity, which is so marked a feature with deprivation of calcium occurs typically only after five or ten minutes of potassium deprivation. The most important immediate change at zero concentration of K is an increase in tonus. This increase in tonus

persists for a time (10 to 15 minutes) and later decreases (one-half hour), accompanied by decrease of amplitude.

The partial or complete restoration of potassium after deprivation of this ion always results in a very characteristic temporary inhibition and often complete cessation of the contractions. The addition of one-fourth the normal amount of potassium to a solution containing none produces a cessation and fall of tone similar to that occurring after return to Locke but somewhat less in degree; after a time (3 to 5 minutes) the beats are resumed and the tone rises (fig. 8, $\frac{1}{4}$ K). The second addition of K, to one-half the normal concentration, frequently causes a similar response. However, the fall of tone is less and the cessation of spontaneous activity is less prolonged than following the addition of one-fourth the normal amount of potassium. The fall of tone and temporary inhibition of contractions is not noted or is only slight on going from one-half to three-fourths and thence to normal concentration of potassium; but the restoration is still complicated by the after-effects of the K deprivation-restoration phenomena, so that the changes in ascending from zero to the normal concentrations are not the simple quantitative reciprocals of the descending concentrations (fig. 1, K).

The significant phenomenon following ascending concentrations of K above normal is progressive rise of tonus, the diminished diastolic relaxation leading to decreased amplitude, and this to increased rate (fig. 1, K). Periodicity appears with one and one-half times the normal concentration of K, even if the osmotic pressure is compensated by decrease of Na concentration (fig. 9).

D. Responses of intestinal segments to alteration of Na concentration. The third of the important cathions of Locke solution, sodium, was studied in the same manner as the calcium and potassium. Alterations were made as before by increments and decrements of one-fourth the normal concentration, whilst the osmotic pressure was maintained nearly constant by the addition of isotonic glucose solution (5.1066 per cent equals 0.9 per cent NaCl). Calcium and potassium were present in normal amounts.

The range of concentration studied was from normal to zero and from zero to two and one-fourth times the normal concentration.

The principal and most constant changes in function resulting from descending concentrations of Na are in the amplitude and tonus (fig. 1, Na). There is progressive decrease of amplitude and a smooth increase of tone on going from one to one-fourth the normal concentration of Na. The direction of change is similar to that resulting from K reduction.

The main phenomena of complete Na deprivation consist in decreased amplitude and increased tone, i.e., an exaggeration of the phenomena produced by decreasing from normal to one-fourth normal concentration The decrease of amplitude and the rise of tone are in the same direction as results from potassium deprivation but much greater in degree.

Ascending concentrations of Na from zero to normal produce functional alterations which are practically the reciprocal of those resulting from reduction of Na from one to zero concentration. There is a progressive decrease of tone and smooth increase of amplitude. The rate is little changed up to a concentration of three-fourths the normal amount of Na; going from three-fourths to normal, the rate is slowed.

Ascending concentrations of Na above the normal involve considerable osmotic changes which would complicate the phenomena to such a degree that they need not be discussed in this place.

E. Response following alteration of all three ions (Ca, Na, K) simultaneously. All three ions (Ca, K, Na) were altered simultaneously by decrements and increments of one-fourth the normal concentration whilst the osmotic pressure was maintained constant by the addition of isotonic glucose solution. The range of concentrations studied was from 1 to zero and from zero to 1 (1 refers to the normal concentration of the ions in Locke). The effects are illustrated in figure 1, Na, Ca and K.

Descending from normal to one-fourth concentrations the principal effect is marked reduction of the amplitude, similar to the reduction which occurs with K and Na and contrary to that which occurs on partial deprivation of Ca. The rate is somewhat slowed as with Ca reduction while the tone is scarcely altered, so that the depressor action of the Ca reduction has neutralized the pressor action of the Na and K reduction.

Change to zero concentration of Ca, Na and K results in cessation of spontaneous activity and rise of median tone similar to Na deprivation. There is no primary fall of tone as occurs with reduction of Na alone to zero concentration.

Ascending concentrations produce functional alterations which are reciprocal to those resulting from descending concentrations: progressive increase of rate and of amplitude. The tone falls slightly on going from 0 to one-fourth normal concentration of these ions: additions above this have no effect on the tone.

TABLE 1

Motor response of Magnus intestines to ions

+ = stimulation; − = depression; 0 = no change.

A. Descending concentrations 1 to $\frac{1}{4}$

REDUCTION OF	AMPLITUDE	RATE	TONE
Ca	+	−	−
K	≡	+	+
Na	≡	0	+
Ca, K	+	−	− = Ca predominates
Ca, K, Na	≣	−	0 = Na, K predominates

TABLE 1—*Concluded*

B. Deprivation

	AMPLITUDE	RATE	TONE	PERIODICITY
Ca	$\overline{\underline{\equiv}}$	?	0	++ +
K	$-$	?	+	+
Na	$\overline{\underline{\equiv}}$	?	+ ++	0
Ca, K	$\equiv$	?	++	++ (1)
Ca, K, Na	$\overline{\underline{\equiv}}$	?	++	0 (2)

(1) Ca predominates in amplitude and periodicity; K in tone.
(2) Na predominates.

C. Restoration

Ca = reciprocal
K = temporary inhibition followed by spasticity
Na = reciprocal

Ca and K = depression followed by recovery (K tempered by Ca)
Ca, K, Na = reciprocal (K neutalized by Na and Ca)

D. Increase above normal

	AMPLITUDE	RATE	TONE	PERIODICITY
Ca	=	++	++	$-$
K	$\equiv$	+	++	+
Ca + K	$\equiv$	+	++	0

Note that K and Ca appear to be synergistic when their concentrations exceed the normal; and antagonistic when their concentration lies below the normal.

E. Integration

Na: increases amplitude, little effect on tone and rate
Ca: increases tone and rate, decreases amplitude
K: below normal, similar to Na
above normal, similar to Ca

Complete deprivation of any or all ions decreases amplitude, but least when K alone is decreased.

The tone rises markedly for deprivation of Na, less for K, not for Ca.

Ca deprivation produces marked periodicity, K much less, Na none.

K deprivation also has a peculiar after-effect, i.e., inhibition on adding K

SUMMARY

The functional changes arising from alteration of the concentration of the important cathions (Ca, Na, K) in Locke solution are summarized for convenience and for the sake of comparison in table 1.

Fractional deprivation and addition of the three important cathions, Ca, Na, K in Locke solution, carried out singly and simultaneously, show that these ions have the following effects on the functions of the smooth muscle of the small intestine of the rabbit:

Sodium increases the amplitude, whilst it has little effect on the tone or the rate of contractions; calcium increases the tone and rate of contractions and decreases the amplitude; potassium in concentrations below normal has effects like sodium, and antagonistic to calcium; above the normal concentration it increases the tone and decreases amplitude, synergistic with calcium, whilst tending to induce periodicity.

Potassium deprivation also has a peculiar after-effect, consisting in inhibition and subsequent tendency to spasticity when this ion is added.

The effects following simultaneous alteration of cathion concentration, in which only the amount of the ions was changed seems not to have been extensively investigated for mammalian intestinal smooth muscle (4), (5), (6). The results herein reported show that the amount as well as the ratio of ions must be considered important in maintaining normal functional activity.

The changes which appear in the form of periodic contractions, especially with deprivation of Ca, K or both of these ions, may be confusing if these are not recognized as qualitative. We doubt whether these qualitative alterations have so far been sufficiently emphasized, although Tezner and Turolt (7) mention irregularities in the contractions of human gastric segments subsequent to complete Ca deprivation.

It is of interest that the complete deprivation of Ca has the most pronounced effect in inducing periodicity and that K deprivation, as well as complete removal of Ca and K results in the appearance of these irregularities less promptly, while removal of Na causes no such response.

The author gratefully acknowledges the advice of Dr. Torald Sollmann and Dr. W. F. von Oettingen in carrying out this work. To Doctor Sollmann particularly, my thanks are due for help in the interpretation of the results and in revising the manuscript.

BIBLIOGRAPHY

(1) Sollmann, T. Manual of pharmacology. 3rd ed., 1926, W. B. Saunders Co., Philadelphia.

(2) Sollmann, T., W. F. von Oettingen and Y. Ishikawa. This Journal, 1928, lxxxv, 118.

(3) Sollmann, T. and W. F. von Oettingen. This Journal, 1928, lxxxvii, 293.

(4) Clark, A. J. Journ. Pharm. Exper. Therap., 1921–22, xviii, 423.
(5) Rosenmann, M. Zeitschr. f. d. ges. Exper. Med., 1922, xxix, 334.
(6) Magee, H. E. and C. Reid. Journ. Physiol., 1927, lxiii, 97.
(7) Tezner, O. and M. Turolt. Zeitschr. f. d. ges. Exper. Med., 1921, xxiv, 1.

THE CEREBRAL CIRCULATION

X. The Action of Histamine

HENRY S. FORBES, HAROLD G. WOLFF and STANLEY COBB

From the Department of Neuropathology, Harvard Medical School, Boston

Received for publication February 18, 1929

It has been found that the blood supply of the brain, or more exactly, the caliber of its vessels, is regulated by several distinct mechanisms (1) (2). Of these one of the most significant appears to be regulation through changes in the chemical composition of the blood. For the purposes of this paper two examples may serve as illustrations of physiological states which bring about such chemical changes, namely: fluctuations in concentration of the normal products of metabolism (notably carbon dioxid), and accumulations of abnormal substances (histamine-like compounds). Increasing tensions of carbon dioxid have been found to be accompanied by increasing degrees of dilatation of the blood vessels of the pia mater, in cats (3). The present report deals with the effect of histamine hydrochlorid on these vessels.

Intravenous injections of histamine have already been made by Lee (4), who observed the pial vessels, but noticed no change in vessel diameter. He made, however, no actual measurements of the vessels and his apparatus did not permit the direct application of the drug to the brain surface beneath the cranial window. In human beings Harmer and Harris (5) have found that headache, throbbing, flushing of the face, and other symptoms follow intravenous injection of histamine, and Weiss and Lennox (6) have found a rise in cerebrospinal fluid pressure in 60 non-anesthetized patients to whom small amounts of histamine had been given intravenously. No appreciable change in arterial or in venous pressure occurred in these cases, owing probably to the small size of the dose. Weiss has also made the following interesting observation. During a head operation on a patient under luminal and paraldehyde narcosis (rectal), a small dose of histamine was given intravenously. Weiss observed a striking increase in the volume of the brain, so that the cortex bulged upwards into the cranial defect, the pulsations of the brain were greatly increased and the brain surface became flushed.

We have employed a technique (previously described (7)) which allows microscopic observation, measurement and photography of the pial blood vessels through a specially designed cranial window; we have thus studied

the effect of histamine hydrochlorid when applied locally to the surface of the brain, injected into a leg vein or into the carotid artery. Two prepara-

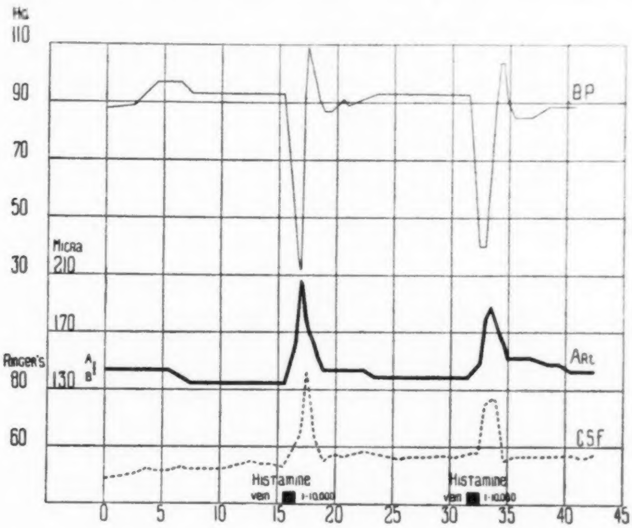


Fig. 1. *Experiment I. Histamine. Intravenous.* Anesthesia, amytal, 30 cc. 1 per cent intraperitoneally. Weight of cat, 3.7 kilo.

After a 13 minute observation period 0.5 cc. of a 1–10,000 solution of histamine (Pfanstiehl) was injected in 15 seconds into the femoral vein. The pial artery dilated 53.3 per cent. Sixteen minutes later 0.5 cc. of a 1–10,000 solution of histamine was again injected intravenously. The artery dilated 35.6 per cent, and recovered its original size more slowly.

In this and the following charts ordinates represent millimeters of mercury, microns and millimeters of Ringer's solution respectively. Abscissas represent time in minutes. Observations of systemic arterial, venous and cerebrospinal fluid pressures and diameters of the pial artery were made at one minute intervals or less in all instances. The solid areas or arrows at the bottom of the charts indicate periods of injection. The broad line indicating the diameter of the pial artery is so plotted that its upper edge records the correct measurements and time relations. The narrow line represents the femoral artery pressure, the broken line, the cerebrospinal fluid pressure, and the line broken by circles (in fig. 3), the saphenous venous pressure. The arrow *AB* (9 microns) represents an extent of change in arterial diameter which could be accurately measured. A change of half this extent is of doubtful validity.

tions of the drug were used, histamine (Pfanstiehl[1]) and Imido-"Roche".[2] Dilutions of 1–1,000, 1–10,000 and 1–100,000 were freshly made up in

[1] Special Chemical Co., Highland Park, Illinois.

[2] Hoffmann LaRoche Chemical Works, New York.

Ringer's solution, the intravenous dosage ranging from 0.003 mgm. to 0.47 mgm. per kilo.

Twenty-one experiments were carried out in ten cats anesthetized with amytal (iso-amyl-ethyl barbituric acid).

In every instance, save one, histamine caused dilatation of all visible blood vessels in the pia, from arteries 288 microns in diameter down to

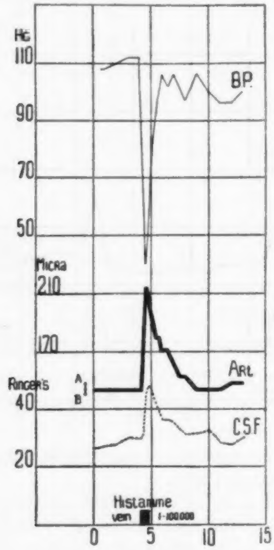


Fig. 2

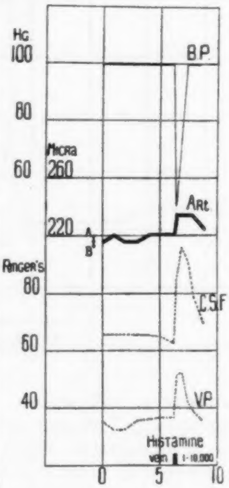


Fig. 3

Fig. 2. *Experiment II. Histamine. Intravenous.* Anesthesia, amytal, 27.5 cc. 1 per cent intraperitoneally. Weight of cat, 3.05 kilo.

After a control period of 4 minutes 1 cc. of a 1–100,000 solution of histamine (Imido-"Roche") was injected during 45 seconds into the femoral vein. The pial artery dilated 50 per cent.

Fig. 3. *Experiment III. Histamine. Intravenous.* Anesthesia, amytal, 33 cc. 1 per cent intraperitoneally. Weight of cat, 3.6 kilo.

(Venous pressure was recorded from the central portion of the ligated saphenous vein.) After a short period of observation 1 cc. of a 1–10,000 solution of histamine (Imido-"Roche") was injected into the brachial vein in 15 seconds. The pial artery dilated 6.3 per cent.

vessels of capillary size. After intravenous injection the cerebral vascular dilatation was accompanied by a fall in systemic arterial (figs. 1 and 2) and a rise in systemic venous pressure and in cerebrospinal fluid pressure (fig. 3). Occasionally a slight transient narrowing of the pial vessels immediately preceded the dilatation. Sometimes also a quick fall in

venous or in cerebrospinal fluid pressure, coincident with increased depth of respiration, preceded by a few seconds the greater rise in these pressures.

When histamine was applied locally to the brain surface no significant changes in blood pressure or in cerebrospinal fluid pressure took place, yet in every instance the pial vessels bathed in the histamine solution dilated sharply (figs. 4 and 5). As mentioned above, in one animal constriction followed the intravenous injection, but in this instance the pial vessels under observation already had been dilated by a previous local application of the drug to the brain surface beneath the window (fig. 5). It is probable that the "constriction" here was merely a passive collapse of the vessel walls accompanying the abrupt fall in arterial pressure. Moreover,

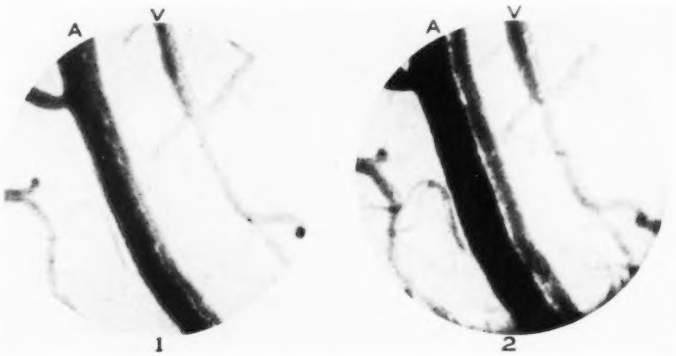


Fig. 4. *Experiment IV. Pial blood vessels before and after histamine. Local.* 1, before histamine. 2, after histamine: 3½ minutes after local irrigation (over the surface of the brain beneath the cranial window) of 2.5 cc. of a warm 1–10,000 solution of histamine (Pfanstiehl) in Ringer's solution. Dilatation both of arteries and veins is evident.

Both photographs—exposure, one second; magnification, 20 ×. *A* = Artery. *V* = Vein.

the cerebrospinal fluid pressure rose, although this intravenous injection, following the local application of histamine, caused a narrowing of the pial vessels in the area beneath the window. In all probability this rise was due to the dilator action of the intravenous histamine on the cerebral vessels generally throughout the brain, since the vast majority of these vessels had not been affected by the previous local application of the drug.

Our method of measuring cerebrospinal fluid pressure was the same as that used by Lee, but the experimental conditions differed in one respect —Lee used ether anesthesia, whereas we used amytal. In order to test the importance of this factor five cats were anesthetized with ether and

eleven experiments with cerebrospinal fluid pressure measurements were carried out. Also two experiments were made in cats under both amytal and ether. Our results were now in agreement with those obtained by Lee. Under ether the cerebrospinal fluid pressure fell after intravenous histamine in every case. The pial arteries in five trials in two animals under ether showed narrowing four times and slight dilatation once (figs. 6 and 7). Local application of histamine to the brain surface of one of these etherized

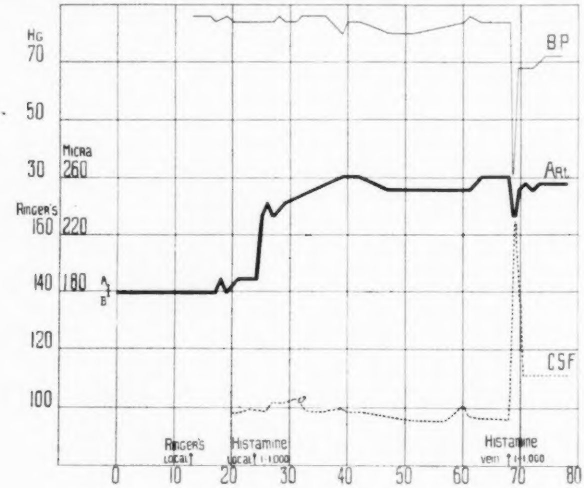


Fig. 5. *Experiment V. Histamine. Local followed by intravenous.* Anesthesia, amytal, 22 cc. 1 per cent intraperitoneally. Weight of cat, 2.1 kilo.

After a short period of observation Ringer's solution was irrigated over the surface of the brain beneath the cranial window. No change in size of the pial artery was noted. Six minutes later a very slight dilatation occurred, but this probably was due to some unknown factor. Eleven minutes later 3 cc. of a warm 1–1,000 solution of histamine (Pfanstiehl) was irrigated in similar manner over the surface of the brain. The artery dilated 42.9 per cent. The lack of change in either systemic arterial or cerebrospinal fluid pressures is to be noted. After 44 minutes 0.25 cc. of a 1–1,000 solution of histamine was injected into the femoral vein. The dilated pial artery now constricted 10.3 per cent with the fall in systemic arterial pressure.

animals caused local dilatation of the same pial vessels which had reacted only slightly—twice becoming narrower—after the intravenous dose. This powerful effect of locally applied histamine was probably due to the greater concentration of the drug when irrigated beneath the window, than when it was injected intravenously and thus diluted by the blood. It was found, too, that ether itself caused dilatation of the pial vessels and a great rise in cerebrospinal fluid pressure (fig. 8). This dilatation would tend to lessen the subsequent effect of the (diluted) histamine.

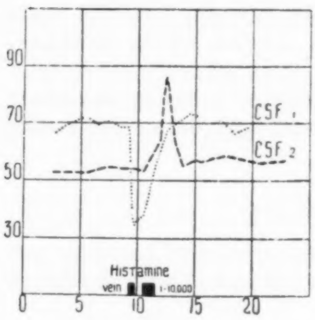


Fig. 6

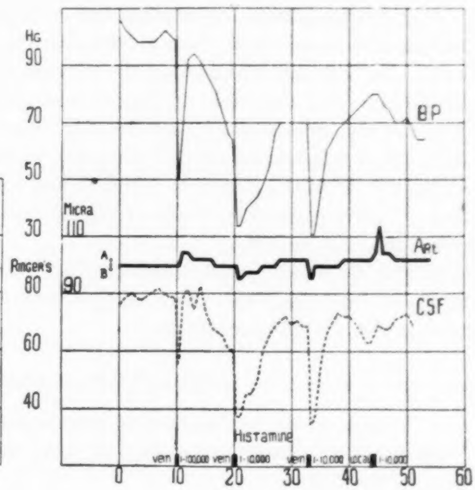


Fig. 7

Fig. 6. *Experiments II and VI. Histamine. Intravenous.* Anesthesia (II), 33 cc. 1 per cent amytal, intraperitoneally. Weight of cat, 3.6 kilo. Anesthesia (VII), ether, inhalation.

Figure illustrating the opposite effect upon cerebrospinal fluid pressure in cats under 1, ether anesthesia; 2, amytal anesthesia, of a 1–10,000 solution of histamine, the dotted line representing the ether animal, the broken line the amytal.

Fig. 7. *Experiment VI. Histamine. Intravenous followed by local anesthesia. Ether* inhalation.

After a control period of $9\frac{3}{4}$ minutes 1 cc. of a 1–100,000 solution of histamine (Imido-"Roche") was injected during 30 seconds into the femoral vein. The pial artery dilated only 10 per cent. Ten minutes later a 45 second injection of 1 cc. of a 1–10,000 solution of histamine (Imido-"Roche") was given intravenously. The pial artery constricted 10 per cent. Thirteen minutes later 1 cc. of a 1–10,000 solution was injected intravenously, during 25 seconds. The artery constricted 15 per cent. Six minutes later 2 cc. of a 1–10,000 solution was irrigated over the surface of the brain beneath the window. The pial artery now dilated 23.8 per cent. (For discussion, see paper.)

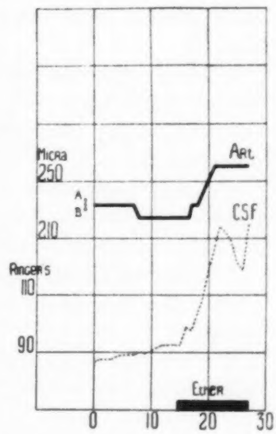


Fig. 8

Fig. 8. *Experiment VII. Ether following amytal anesthesia.* Anesthesia, amytal, 30 cc. 1 per cent intraperitoneally. Weight of cat, 3.6 kilo.

After an observation period of $14\frac{1}{2}$ minutes ether was inhaled through the tracheal cannula. The cerebrospinal fluid pressure rose immediately. The pial artery dilated 16 per cent in $7\frac{1}{2}$ minutes, and remained dilated despite the changes in cerebrospinal fluid pressure.

It might be possible to explain the cerebral vasodilator effect of intravenous histamine simply as a mechanical result of the rise in venous pressure. To test this supposition the venous pressure was raised artificially by intravenous injection of Ringer's solution. The cerebrospinal fluid pressure rose coincidently, but the pial vessels did not dilate; on the contrary, they constricted (8). The height to which the venous pressure was raised exceeded the height to which it had risen following histamine injection. The suggested mechanical explanation, therefore, of the vasodilatation could not be valid.

It may be worth mentioning here that the difficulties due to hemorrhage from the bone and from the meninges were strikingly greater under ether than under amytal anesthesia.

SUMMARY AND CONCLUSION

1. Vessels of the brain react to intravenous injection of histamine in a very different manner, depending on whether the animal is under ether or amytal anesthesia.
2. Under amytal the vessels of the brain dilate (following intravenous injection of histamine) and the cerebrospinal fluid pressure rises. These same results occur after small doses of histamine in non-anesthetized man (6).
3. Under ether the vessels of the brain are already dilated, and little if any further dilatation is seen following the intravenous injection of histamine. On the contrary they often become narrower and the cerebrospinal fluid pressure falls.
4. Local application of histamine to the surface of the brain always results in dilatation of the pial vessels, without noticeably affecting intracranial or systemic vascular pressures.
5. Intravenous injection of histamine (in animals under amytal) causes great pial artery dilatation in spite of a coincident fall in systemic arterial pressure.
6. Chemical changes in the blood may be and often are more powerful than alterations in blood pressure as a means of regulating the caliber of the pial blood vessels in mammals.

BIBLIOGRAPHY

(1) Forbes, H. S. and H. G. Wolff. Arch. Neurol. and Psychiat., 1928, xix, 1057.
(2) Wolff, H. G. and H. S. Forbes. Arch. Neurol. and Psychiat., 1928, xx, 73.
(3) Forbes, H. S., H. G. Wolff and W. G. Lennox. Not yet published.
(4) Lee, F. C. This Journal, 1925, lxxiv, 317.
(5) Harmer, I. M. and K. E. Harris. Heart, 1926, xiii, 381.
(6) Weiss, S. and W. G. Lennox. Personal communication.
(7) Forbes, H. S. Arch. Neurol. and Psychiat., 1928, xix, 751.
(8) Forbes, H. S. and S. Cobb. To be published.

I. THE RELATIVE AMOUNTS OF PRESSOR AND RENAL ACTIVITY IN "VASOPRESSIN" AND "OXYTOCIN"

II. SOME EVIDENCE INDICATING THE PRESENCE OF A THIRD OR RENAL HORMONE IN THE POSTERIOR LOBE OF THE PITUITARY GLAND

W. B. DRAPER

From the Department of Physiology, University of Colorado School of Medicine, Denver

Received for publication February 19, 1929

The long drawn out controversy concerning the unity or multiplicity of the active principles responsible for the three major physiological responses following the administration of an extract of the posterior lobe of the pituitary gland (pressor, diuretic-antidiuretic and oxytocic) appears now to have been settled.

Historical. The first acceptable evidence of the presence of two different hormones in this extract was probably that presented by Dudley (1919). This author showed that normal butyl alcohol removed from an aqueous extract of the lobe all its oxytocic activity but left behind in the aqueous layer 40 per cent of its pressor activity. He took this as evidence that the pressor and oxytocic activities of the extract are due to separate chemical individuals. Schlapp (1925) repeated Dudley's work and confirmed his findings. Neither of these authors attempted to determine the partition between the two layers of the diuretic-antidiuretic activity.

Dudley's work was again repeated by the writer (1927) with substantially the same results and in addition it was found that the diuretic-antidiuretic activity followed closely the pressor activity during normal butyl alcohol extraction. The writer also showed that complete recovery, within the limits of experimental accuracy, of the active principles could be obtained following the extraction. This eliminated any possibility that the experimental results were due to injury to the molecule.

A number of writers have presented evidence showing that the pressor and oxytocic activities disappear simultaneously when the extract is subjected to heat, tryptic digestion, alkaline hydrolysis and hydrochloric acid decomposition and that these activities also have equal diffusion rates. These results were interpreted by these writers as presumptive evidence of the unitarian nature of the hormones.

The chief support of the unitarian theory, however, was the work of Abel, Rouiller and Geiling (1924). These authors claim to have isolated from the posterior lobe of the gland a purified tartrate having an oxytocic titer of from 500 to 1000 times that of histamine acid phosphate in which the oxytocic, pressor and diuretic-antidiuretic activities of the gland are preserved in their original proportions. This claim, however, was probably due to a misinterpretation of their results since their data indicate, as pointed out by the writer (1927), that only 0.1 per cent or less of the original pressor and diuretic-antidiuretic activities still remained in the tartrate.

The recent publication by Kamm, Aldrich, Grote, Rowe and Bugbee (1928) reports the almost complete separation, by means of repeated fractional precipitations, of the oxytocic and pressor activities. Preparations embodying their results are now on the market under the trade names, "oxytocin" and "vasopressin."

In a subsequent paper Bugbee and Simond (1928) investigated the distribution of the diuretic-antidiuretic activity between these two fractions. These workers employed rabbits which were fed on oats and green food with plenty of water always available. The experiments were conducted under urethane, amytal or chloral hydrate anesthesia and the urine was collected by means of a bladder fistula. Using this technique they found that the oxytocic principle had very little, if any, diuretic-antidiuretic action and that the pressor principle had a powerful diuretic-antidiuretic action. It will be noted that these authors employed for their assay animals which had ingested an unknown but probably large amount of water and, further, that the excretion of chlorides subsequent to the injection was not observed.

It was the experience of the writer (1927), while endeavoring to develop a method for the bio-assay of the renal activity, that the volume response to pituitary extract could only be relied upon when great care was taken to stabilize the water content of the animal. In addition it was found that the changes in the rate of chloride excretion subsequent to the injection are a valuable check on the changes in urinary flow. It is also very desirable to avoid anesthesia since this also influences the renal response.

Experimental procedure. The pressor activities of "oxytocin" and "vasopressin"[1] were determined on spinal cats by the method proposed by Hogben, Schlapp and McDonald (1924). This method has the advantage of not requiring anesthesia. The injections are made at intervals of one hour.

The renal activity of "oxytocin" and "vasopressin" was determined by the method suggested by the writer (1927). All determinations in any

[1] The material for this assay was furnished by the kindness of the manufacturers, Parke, Davis & Company.

one series of experiments are made upon the same bladder fistula dog. Before each experiment a flanged glass cannula is inserted into the bladder in order to prevent pocketing of the urine. The urine is collected during the experiment by placing the animal on a table with the fistula over a funnel set in the top of the table. Under these conditions the urine can be collected as formed with great accuracy. One injection only is made in the course of a single experiment. This is important since the effect of even a small dose of the extract on the excretion of chlorides can still be observed long after the urine flow has returned to normal. It is essential, in order to obtain consistent results, to establish uniform conditions, especially with regard to the water and salt intake of the animal. This

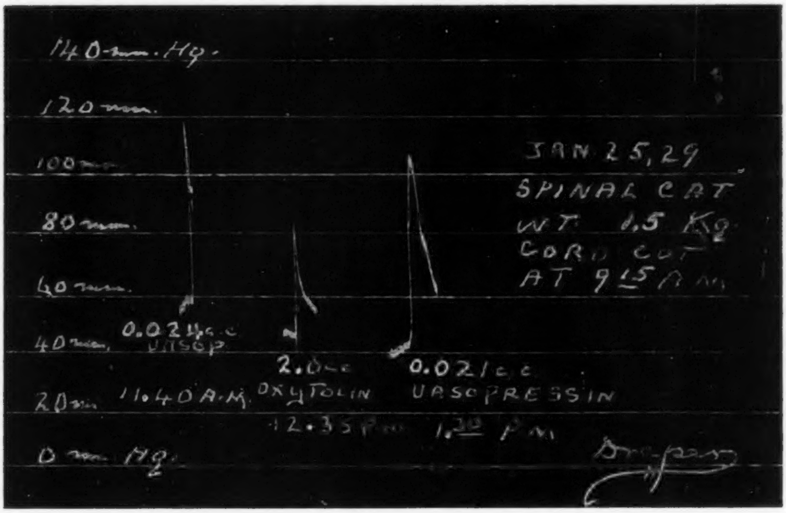


Fig. 1

may best be done by depriving the animal of all food and water for the 24 hours preceding the experiment. The diet used in these experiments was "Kippy," a canned commercial ration composed of horse meat and vegetables. Control of the diet was found to be important since an uncertain diet, such as kitchen scraps, results in control urines which differ greatly in their chloride content. A great advantage obtained when this technique is employed is the accurate control rate of urine excretion which is established. This is, of course, essential to any attempt at bio-assay.

Under these conditions the urinary output of the animal is responsive to minute doses of the principle. Using standard pituitary powder it was found that a definite diuresis and an even greater increase in the chloride

excretion could be obtained following the intravenous injection of doses which produce a submaximal rise in blood pressure. In the course of a large number of experiments designed to test the accuracy of this method it was found that the best results could be obtained by injecting a series of descending doses until the minimal effective diuretic dose was determined. The animal responds with the greatest accuracy when the amounts injected are just above this dose. With these small doses the water-deprived animal often shows an initial antidiuresis lasting from one to three minutes but a prolonged antidiuresis is never seen. In general, the length of the antidiuretic period is roughly proportional to the amount of water recently ingested by the animal. Spontaneous changes in the urine flow and abnormal responses seldom occur. A further safeguard against volume changes not due to pituitary activity is found in frequent determinations of the chloride excretion. Pituitary diuresis or antidiuresis is always accompanied by a considerable increase in chloride excretion.

In the opinion of the writer this method, although time-consuming, is probably the only one, at present available, which yields results accurate to within 25 per cent.

Results of assays. The accompanying graph indicates that the pressor activity per cubic centimeter of "vasopressin" is more than 100 times that of "oxytocin." In fact, "oxytocin" has so weak a pressor action that its exact pressor assay is difficult in view of the large intravenous dose which is required to cause any considerable rise in blood pressure. Similar results were obtained in each of the experiments conducted. An interesting feature of these assays was the constant tendency of "oxytocin" to cause a fall in blood pressure immediately following the injection. This is not seen after "vasopressin." In several assays the pressor activity of "vasopressin" was measured against that of standard pituitary powder and it was found to be somewhat in excess of the 20 pressor units per cubic centimeter claimed by the manufacturer.

The accompanying table gives in detail the results of the renal assay. It will be seen that "oxytocin" is by no means free from renal activity. In fact, it contains one-half as much renal activity per cubic centimeter as "vasopressin." The table also indicates the renal response to 0.2 mgm. of standard powder which is the equivalent of $\frac{1}{25}$ cc. of liquor pituitarii of the U.S.P. X. The renal activity of "oxytocin" is, therefore, rather more than one-half and that of "vasopressin" is approximately equal to that of equal volumes of the official liquor pituitarii.

A notable feature of the renal assays which was observed but which, owing to its briefness is not shown clearly in the table, was the greater tendency of "vasopressin" to produce an initial antidiuresis. Small doses of "oxytocin" rarely cause a noticeable preliminary antidiuresis while this

RENAL ASSAY OF OXYTOCIN AND VASOPRESSIN ON A BLADDER FISTULA DOG. PREPARATION OF ANIMAL FOR EXPERIMENT: NO FOOD, NO WATER FOR 24 HOURS BEFORE EXPERIMENT. DIET: "KIPPY."

	Control Period			Dose	Following Injection				
Urine in 10 minute periods	1.3 cc.	1.3 cc.	1.4 cc.	0.10 cc. Oxytocin	2.3 cc.	1.9 cc.	1.8 cc.	1.6 cc.	1.5 cc.
Volume of urine per minute	0.133 cc.				0.230 cc.	0.185 cc.		0.155 cc.	
NaCl per cent	0.075 %				0.609 %	0.270 %		0.226 %	
NaCl per minute	0.10 mg.				1.40 mg.	0.50 mg.		0.35 mg.	
Urine in 10 minute periods	0.9 cc.	0.9 cc.	0.8 cc.	0.07 cc. Oxytocin	1.5 cc.	1.4 cc.	1.3 cc.	1.0 cc.	1.0 cc.
Volume of urine per minute	0.087 cc.				0.150 cc.	0.135 cc.		0.100 cc.	
NaCl per cent	0.077 %				0.400 %	0.148 %		0.100 %	
NaCl per minute	0.07 mg.				0.60 mg.	0.20 mg.		0.10 mg.	
Urine in 10 minute periods	0.8 cc.	0.8 cc.	0.8 cc.	0.06 cc. Oxytocin	1.0 cc.	0.8 cc.	0.6 cc.	0.7 cc.	0.7 cc.
Volume of urine per minute	0.08 cc.				0.100 cc.	0.070 cc.		0.070 cc.	
NaCl per cent	0.100 %				0.280 %	0.214 %		0.129 %	
NaCl per minute	0.08 mg.				0.28 mg.	0.15 mg.		0.09 mg.	
Urine in 10 minute periods	0.9 cc.	0.7 cc.	0.8 cc.	0.05 cc. Oxytocin	0.8 cc.	1.0 cc.	0.8 cc.	1.0 cc.	0.8 cc.
Volume of urine per minute	0.080 cc.				0.080 cc.	0.090 cc.		0.090 cc.	
NaCl per cent	0.063 %				0.175 %	0.222 %		0.078 %	
NaCl per minute	0.05 mg.				0.14 mg.	0.20 mg.		0.07 mg.	
Urine in 10 minute periods	1.0 cc.	0.9 cc.	0.9 cc.	0.04 cc. Vasopressin	0.6 cc.	1.5 cc.	1.4 cc.	1.4 cc.	1.0 cc.
Volume of urine per minute	0.093 cc.				0.060 cc.	0.145 cc.		0.120 cc.	
NaCl per cent	0.100 %				0.233 %	0.496 %		0.375 %	
NaCl per minute	0.09 mg.				0.14 mg.	0.72 mg.		0.45 mg.	
Urine in 10 minute periods	0.9 cc.	0.7 cc.	0.8 cc.	0.035 cc. Vasopressin	1.1 cc.	1.1 cc.	1.2 cc.	0.9 cc.	0.8 cc.
Volume of urine per minute	0.080 cc.				0.110 cc.	0.115 cc.		0.085 cc.	
NaCl per cent	0.108 %				0.273 %	0.391 %		0.247 %	
NaCl per minute	0.09 mg.				0.30 mg.	0.45 mg.		0.21 mg.	
Urine in 10 minute periods	0.8 cc.	0.8 cc.	0.7 cc.	0.029 cc. Vasopressin	0.7 cc.	0.9 cc.	0.7 cc.	0.8 cc.	0.8 cc.
Volume of urine per minute	0.077 cc.				0.070 cc.	0.080 cc.		0.080 cc.	
NaCl per cent	0.122 %				0.286 %	0.387 %		0.275 %	
NaCl per minute	0.09 mg.				0.20 mg.	0.31 mg.		0.22 mg.	
Urine in 10 minute periods	0.9 cc.	0.8 cc.	0.9 cc.	0.20 mgm. Standard Pituitary Powder	1.3 cc.	1.2 cc.	0.8 cc.	0.8 cc.	0.8 cc.
Volume of urine per minute	0.087 cc.				0.130 cc.	0.100 cc.		0.080 cc.	
NaCl per cent	0.269 %				0.508 %	0.340 %		0.312 %	
NaCl per minute	0.23 mg				0.66 mg.	0.34 mg.		0.25 mg.	
Urine in 10 minute periods	0.9 cc.	0.8 cc.	0.9 cc.	0.18 mgm. Standard Pituitary Powder	1.2 cc.	0.8 cc.	0.9 cc.	1.0 cc.	0.8 cc.
Volume of urine per minute	0.087 cc.				0.120 cc.	0.085 cc.		0.090 cc.	
NaCl per cent	0.092 %				0.600 %	0.353 %		0.222 %	
NaCl per minute	0.08 mg.				0.72 mg.	0.30 mg.		0.20 mg.	

condition following equivalent doses of "vasopressin" often lasts as long as five minutes. With larger doses than were used in this assay this feature was even more marked. This phenomenon is probably due to the influence of the large amount of pressor activity in "vasopressin." This activity may, by disturbing the circulation in the kidney or by causing contraction of the ureters, delay the onset of the diuresis. The rise in chloride concentration which is so characteristic of pituitary diuresis is, however, marked during the period of antidiuresis.

DISCUSSION. It is apparent from the data presented that the normal ratio of renal to pressor activity as exhibited by extracts made from standard powder is greatly disturbed in "oxytocin." An amount of "oxytocin" exhibiting a pressor activity equal to that of a given amount of the standard powder contains at least twenty-five times its renal activity. The normal ratio is also disturbed in "vasopressin" since it contains only one-half the normal amount of renal activity. If the renal activities of pressor equivalents of "oxytocin" and "vasopressin" be compared, it will be seen that "oxytocin" is more than fifty times as active as "vasopressin."

The renal principle of extracts of the posterior lobe is, therefore, in all probability, chemically separate from the pressor and oxytocic principles.

Such a conclusion may be criticised on the ground that some injury to the molecule may have occurred during the chemical manipulations the material has undergone. This is conceivable but not very probable.

It is reasonable to suppose that any injury to the molecule would manifest itself as a loss in activity. The data of Kamm et al (1928) show that complete recovery of the pressor and oxytocic activities was obtained. This precludes the possibility of any injury to these activities during their separation. In this connection it is interesting to observe, as shown by the following table, that the sum of the renal, oxytocic and pressor activities of 2 cc. of "oxytocin" and 1 cc. of "vasopressin" is roughly equal to that possessed by 10 mgm. of standard pituitary powder.

	RENAL ACTIVITY	OXYTOCIC ACTIVITY	PRESSOR ACTIVITY
	mgm.	*mgm.*	*mgm.*
Activities of 2 cc. of "oxytocin" expressed in mgm. of standard powder	5	10	0
Activities of 1 cc. of "vasopressin" expressed in mgm. of standard powder	5	0	10
Total	10	10	10

Since no oxytocic assays were made the oxytocic activities given in the table are those claimed by the manufacturer on the label.

This is in the nature of a preliminary report and further studies on this problem are being made in this laboratory.

SUMMARY

1. The renal activity of "oxytocin" is more than fifty times that of a pressor equivalent of "vasopressin" and twenty-five times that of a like amount of standard pituitary powder.

2. This is taken as indicating the presence of a third or renal hormone in extracts of the posterior lobe of the pituitary gland which is chemically separate from the pressor and oxytocic hormones.

BIBLIOGRAPHY

Dudley, H. W. 1919. Journ. Pharm. Exper. Therap., xiv, 295.

Schlapp, W. 1925. Quart. Journ. Exper. Physiol., xv, 327.

Draper, W. B. 1927. This Journal, lxxx, 90.

Abel, J. J., C. H. Rouiller and E. M. K. Geiling. 1924. Journ. Pharm. Exper. Therap., xxii, 289.

Kamm, O., T. B. Aldrich, I. W. Grote, L. W. Rowe and E. P. Bugbee. 1928. Journ. Amer. Chem. Soc., l, 573.

Bugbee, E. P. and A. E. Simond. 1928. This Journal, lxxxvi, 171.

Hogben, L. T., W. Schlapp and A. D. MacDonald. 1924. Quart. Journ. Exper. Physiol., xiv, 301.

STUDIES IN THE METABOLISM OF THE BILE

IV. The Rôle of the Lymphatics in the Early Stages of the Development of Obstructive Jaundice

CHARLES MAYO, 2nd and CARL H. GREENE

With the technical assistance of Louise Rowley

From The Mayo Foundation and The Mayo Clinic, Rochester, Minnesota

Received for publication April 4, 1929

In every case of jaundice, bile or its characteristic pigment, bilirubin, accumulates in the blood, but the channel by which it enters has given rise to controversy.

Saunders, the first experimental student of the problem, in 1795, ligated the hepatic duct in a dog. Two hours later he noted that the lymphatics were distended with bile-stained fluid and that the serum obtained from blood taken from the hepatic vein was more strongly tinged with bile than that from the jugular vein. Thus it may be seen that Saunders recognized the two chief pathways by which bile may pass from the liver to the blood stream.

The earlier observers were concerned primarily with the time of onset of clinically recognizable jaundice or of the appearance of bile in the blood, lymph or urine. The latter either was observed grossly or was demonstrated qualitatively by some modification of the reaction with nitric acid (Gmelin test) first used by Tiedemann and Gmelin, to show that the staining of the serum or lymph in obstructive jaundice really was due to the presence of bile pigments. In the majority of these experiments, too, the effect of the activity of the gall bladder in delaying the speed with which jaundice develops, with consequent variation in the results, was not clearly recognized.

Various other investigators, and especially Wertheimer and Lepage injected solutions of bilirubin or other materials into the biliary passages and measured the speed with which they appeared in the urine or lymph. This work later was reviewed by Mendel and Underhill who pointed out that although the results of these experiments favor the view that these substances are absorbed directly into the blood stream, it is still an open question whether such experiments can be accepted as reproducing satisfactorily the conditions present in obstructive jaundice.

The van den Bergh test has provided a much more sensitive test for bili-

rubin than the Gmelin or Salkowski tests. In consequence, the emphasis in the search for evidence of the vascular resorption of bile pigment has been shifted from the study of the urine to the study of the blood serum. As a result, the studies of Bloom and of Barron and Bumstead have been much more convincing than some of the earlier ones. The latter have also summarized the histologic evidence, bearing on this problem.

The concept that jaundice is produced by suppression or retention is old. As long as the liver was regarded as the chief site of formation of bilirubin, this concept was not seriously questioned. However, the extrahepatic formation of bilirubin is now generally accepted. Recently Bollman, Sheard and Mann have used the spectrophotometer in the study of the changes in the bilirubin content of serum during the first few minutes after ligation of the common duct and in the study of the relation between the secretory pressure of the bile and the level of serum bilirubin. As a result they have reëmphasized the rôle of functional disturbances in the hepatic cells, with consequent retention of bilirubin in the development of jaundice.

That the bile acids are formed in the liver is now generally accepted (Whipple). The study of the resorption of the bile acids in obstructive jaundice is, therefore, preferable to the study of bilirubin, for the effect of direct retention in the blood stream may be excluded. Fleischl and Kufferath used both the Gmelin and Pettenkofer tests in their experiments and found that the bilirubin and bile acids behaved in a similar manner. Kunkel (1875) also reported the crystallization of bile salts from the lymph. In one experiment 872 mgm. of bile salts were crystallized from 200 cc. of lymph obtained from a dog after ligation of the bile duct. Further experiments along this line were abandoned because of the difficulty in the use of the Pettenkofer test. Recently Aldrich (1928), working in this laboratory, devised a quantitative modification of the test applicable to the analysis of blood or lymph. We have used this method to reinvestigate the changes in the blood and lymph following the production of biliary obstruction.

Experimental methods. All experiments were made on dogs under amytal narcosis. Biliary obstruction was produced by ligation of the cystic and common bile-ducts. Scrupulous care was taken in these experiments to prevent any escape of bile into the peritoneum, for Walters and Bollman have shown that bile is absorbed rapidly from the peritoneal cavity by the lymphatics. One series of animals was kept as a control of the effect of the obstruction alone. In a second series a thoracic duct fistula was established and the lymph drained externally, and in a third series the thoracic duct was ligated at its point of entrance into the jugular vein. Samples of lymph and blood were taken first at intervals of fifteen minutes, and later at intervals of thirty minutes during

the course of the experiment which usually lasted for five hours. The bile acids in the blood and lymph were studied by means of a quantitative modification of the Pottenkofer test; the bilirubin was studied by the van den Bergh test (Greene, Snell, and Walters).

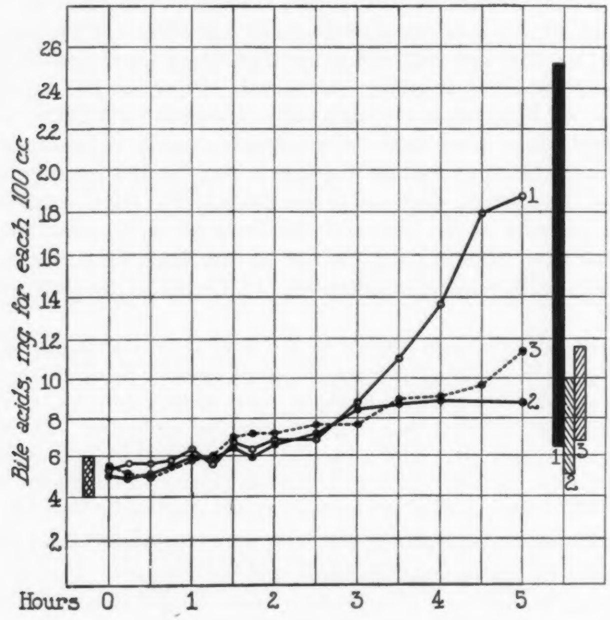


Fig. 1. Comparison of the changes in the bile acid content of the blood: 1, following cholecystectomy, and ligation of the common bile-duct; 2, following cholecystectomy, ligation of the common bile-duct and the production of a thoracic duct fistula; 3, following cholecystectomy and ligation of the thoracic and common bile-ducts. The columns indicate the minimal and maximal values obtained before and at the end of the experiment in each series.

Experimental data. Five experiments were carried out in each series. The accompanying tables present the changes in an illustrative experiment from each. Considerable variations were present in the responses of different dogs and in consequence it is not wise to make too sharp a distinction between the changes observed in each series of experiments. A comparison between the changes of the bile acid content of the blood in these three experiments is shown in figure 1. The range of variation in each experimental series is also indicated on

the figure. The cases reported have been selected to represent as accurately as possible the mean of each experimental series. As such the differences apparently are significant.

When the gall bladder is removed or excluded the changes in the blood stream accord with those previously reported by Snell, Greene and Rowntree (table 1). In general there is a rapid and progressive increase in the bile acid content of the blood as measured by the Pettenkofer reaction. The normal value varies between 4.0 and 6.0 mgm. An increase is demonstrable within thirty minutes and values of 8 to 11 mgm. may be attained at the end of the hour. Two and a half hours after obstruction the readings

TABLE 1

Changes following cholecystectomy and ligation of the common bile-duct

TIME	BLOOD SERUM		
	Van den Bergh direct reaction	Bilirubin	Bile acids
hours		*mgm. per cent*	*mgm. per cent*
0.00	0	0.0	5.1
0.25	0	0.0	5.6
0.50	0	0.0	5.6
0.75	0	0.0	5.8
1.00	0	0.0	6.4
1.25	0	0.0	5.5
1.50	±	0.0	6.7
1.75	±	0.4	6.2
2.00	±	0.5	6.8
2.50	±	0.5	6.8
3.00	±	0.7	8.7
3.50	+	0.9	11.0
4.00	+	1.0	13.7
4.50	+	1.3	17.9
5.00	+	1.4	18.7

varied between 4.5 and 23.2 with an average of 9.8. Five hours after the obstruction these values varied between 6.4 and 25.2 with an average of 15.1. A progressive increase in the serum bilirubin accompanies this change in the bile acids. A delayed or diphasic van den Bergh reaction may appear within fifteen minutes although it is not usual until an hour or an hour and a half has elapsed. It is soon superseded by the direct reaction. Clinical icterus does not appear for twenty-four hours but serum bilirubin of 1.5 mgm. for each 100 cc. is usually present within five hours, the values varying between 0.0 and 5.0 mgm.

A different picture is found when the lymph is removed by a fistula of the thoracic duct (table 2). The lymph from such a fistula rapidly becomes bile-stained after ligation of the common bile-duct. The change in the

bile acid content is most striking; for example, in this experiment the lymph first obtained contained bile acids in a concentration of 12.5 mgm. for each 100 cc. An hour and three-quarters after the ligation of the common bile-duct this had increased to 102.0 mgm. for each 100 cc. These high values were then maintained throughout the experiment or else decreased slightly as the condition of the animal became less satisfactory. A delayed or diphasic van den Bergh reaction was obtained from the lymph from half to one and a half hours after the obstruction to the bile ducts. This was shortly replaced by a direct reaction. The accumulation

TABLE 2

Changes following cholecystectomy and ligation of the common bile-duct in the presence of a thoracic duct fistula

TIME	BLOOD SERUM			LYMPH		
	Van den Bergh direct reaction	Serum bilirubin	Bile acids	Van den Bergh direct reaction	Bilirubin	Bile acids
hours		*mgm. per cent*	*mgm. per cent*		*mgm. per cent*	*mgm. per cent*
0.00	0	0.0	4.9	0	0.0	12.5
0.25	0	0.0	4.8	0	0.0	22.0
0.50	0	0.0	5.1	±	0.8	26.6
0.75	0	0.0	5.5	±	1.7	11.9
1.00	0	0.0	6.0	±	2.4	53.0
1.25	0	0.0	5.9	+	3.5	72.8
1.50	0	0.0	6.7	+	3.6	73.2
1.75	0	0.0	5.9	+	3.7	102.0
2.00	±	0.0	6.6	+	3.8	
2.50	±	0.0	7.1	+	4.8	96.4
3.00	±	0.0	8.5	+	3.8	95.6
3.50	±	0.0	8.7	+	3.7	80.0
4.00	+	0.0	8.8	+	4.6	97.0
4.50						
5.00	±	0.2	8.8	+	4.9	64.2

of pigment was rapid and a bilirubin concentration of 4.0 to 10.0 mgm. was obtained.

This contrast between the changes in the blood and those in the lymph were most striking. The bile acid content of the blood did not change or only increased slightly during these experiments in which there was external lymphatic drainage. Two and a half hours after the obstruction the values varied between 5.7 and 8.1 with an average of 7.0, and readings of 5.7 to 9.8 with an average of 8.0 were found at the end of the observation period. Similarly the development of bilirubinemia was much delayed. A delayed or diphasic van den Bergh reaction was not obtained for one and a half to three hours, and a direct reaction did not appear until after

three and a half to five hours. The amount of bilirubin in the serum in the majority of these experiments was too slight to be measured quantitatively and the maximal value was 0.7 mgm. for each 100 cc. at the end of five hours.

A condition intermediate between these two extremes was produced by the ligation of the thoracic duct at the time the gall bladder was removed and the bile-duct ligated (table 3). There was a slow but progressive increase in the bile acid content of the blood; for example, a value of 11.5 was obtained in the experiment at the end of five hours. The bilirubin likewise increased slowly. A delayed or diphasic van den Bergh reaction was obtained one to one and a half hours after ligation of the duct. The serum bilirubin at the end of five hours varied between 1.3 and 1.4 mgm. for each 100 cc. The changes were more marked than in the presence of a thoracic duct fistula and approached but did not quite equal those in the first series of experiments in which the bile-duct alone was ligated.

TABLE 3

Changes following cholecystectomy and ligation of the thoracic and common bile-duct

TIME	BLOOD SERUM		
	Van den Bergh direct reaction	Bilirubin	Bile acids
hours		*mgm. per cent*	*mgm. per cent*
0.00	0	0.0	5.5
0.25	0	0.0	5.1
0.50	0	0.0	5.0
0.75	0	0.0	
1.00	0	0.0	6.0
1.25	±	0.0	6.1
1.50	±	0.0	7.0
1.75	±	0.0	7.2
2.00	±	0.8	7.1
2.50	±	1.0	7.7
3.00	±	1.2	7.6
3.50	+	1.0	8.9
4.00	+	1.3	9.0
4.50	+	1.4	9.7
5.00	+	1.4	11.5

Comment. A consideration of these data makes it possible to correlate the results obtained by the majority of the previous experimenters. Part of the discrepancies in the early reports dealing with the time at which jaundice developed can be referred to the effect of the gall bladder in delaying the development of intraductal pressure. When the gall bladder is removed, the pressure develops rapidly after ligation of the common

bile-duct. Depending on the sensitivity of the test used, an increase in the serum bilirubin can be recognized in from five minutes (Bollman, Sheard and Mann) to three hours (Saunders, Bloom, Snell, Greene, and Rowntree, Barron and Bumstead). The increase of the bile acid content of the blood is as striking as that of the bilirubin if not more so. In this we confirm the previous studies of Snell, Greene, and Rowntree.

Bile enters the lymphatics rapidly when there is obstruction to the common duct. Bilirubin can be demonstrated in the lymph from a fistula of the thoracic duct within fifteen minutes to an hour (Bloom, Barron and Bumstead) and by two hours is present in great quantity (Saunders, Fleischl, Gerhardt, Wertheimer and Lepage). The changes in the bile acids are even more striking (Fleischl, Kunkel). This external drainage of the lymph delays the accumulation of bilirubin and bile acids in the blood for several hours (Fleischl, Gerhardt, Bloom, Barron and Bumstead) but does not prevent the final development of jaundice (Wertheimer and Lepage, Whipple and King). Ligation of the thoracic duct likewise may delay the appearance of bilirubin or bile acids in the blood stream although to a lesser extent than external drainage (Kufferath, Bloom, Barron, Bumstead), but again does not prevent the eventual development of jaundice (Gerhardt, Wertheimer and Lepage).

Bollman, Sheard, and Mann have stressed the rapidity with which bilirubinemia develops after biliary obstruction, as evidence of functional derangement of the hepatic cells with consequent retention of bilirubin of extrahepatic origin within the blood stream. It is difficult to explain the changes observed by them in any other way. The resorption of bile, however, is an additional factor, at least in the initial stages of obstructive jaundice.

The exact locus of origin of lymph in the liver is unknown. Lymphatic vessels are found in the portal spaces, beside the portal vein and the bile duct, but have not been traced into the hepatic lobule. Merkel held that each vascular capillary was surrounded by a lymphatic space but this is denied by Beale, Mall and others. McIndoe, however, has emphasized that the vascular capillary is surrounded by an extensive felt-work of reticular fibers. This perivascular reticular space, which intervenes between the hepatic cells and the endothelial lining of the capillaries, is continuous with the portal spaces. It is probable, therefore, that tissue fluids may pass along this reticular space to the portal spaces and there find entrance into the lymphatic capillaries. Changes in the chemical composition of the lymph would then indicate corresponding changes in the chemical composition of the tissue fluids.

From this point of view the contrast between the rate of accumulation of bilirubin and bile acids in the blood and lymph is striking. This is particularly true in the case of the bile acids. The concentration of the

latter in the lymph is considerably greater than the maximum found in the blood in cases of biliary obstruction and approaches that ordinarily present in bile from the hepatic ducts. This may indicate a true "parapedesis" or reversal in the polarity of secretion of the hepatic cells in the sense of Minkowski, for Bloom, and Barron and Bumstead have indicated that the bile capillaries are intact during the early stage of obstruction and that rupture of the bile capillaries is not a factor in these changes. On the other hand, the mounting pressure within the bile-ducts would undoubtedly favor the diffusion of bile into the surrounding tissue spaces and lymphatics. The high level of bile acids in the lymph would then be indicative of an approximate equilibrium between the lymph and the contents of the bile capillaries or larger bile passages. In this connection it is noteworthy that after long-continued biliary obstruction the contents of the gall bladder or larger bile-ducts may fail totally to give any test for either bilirubin or bile acids.

If diffusion takes place between the bile capillaries and the lymph or tissue fluids in the reticular spaces then it is probable that similar diffusion may take place between the latter and the adjacent blood capillaries, especially after ligation of the thoracic duct, with resultant lymphatic stasis. These considerations we believe explain the majority of the contradictory results of other investigators regarding the pathway by which the bile reaches the blood in obstructive jaundice.

SUMMARY

Bilirubin and bile acids rapidly accumulate in the blood after the experimental ligation of the cystic and common bile-ducts. The lymph shows an even more rapid accumulation of these two biliary products, and the external drainage of the lymph through a fistula of the thoracic duct delays the changes in the blood stream, but does not prevent the development of icterus. Ligation of the thoracic duct may delay the entrance of bile into the blood stream slightly but not to as great an extent as does a lymphatic fistula.

The results indicate that bile resorption in obstructive jaundice may occur through both vascular and lymphatic channels, although the latter possibly are the more active during the first few hours of obstruction.

BIBLIOGRAPHY

ALDRICH, M. AND M. S. BLEDSOE. 1928. Journ. Biol. Chem., lxxvii, 519.

BARRON, E. S. G. AND J. H. BUMSTEAD. 1928. Journ. Exper. Med., xlvii, 991.

BEALE, L. S. 1889. The liver. London, J. and A. Churchill, p. 55.

BLOOM, W. 1923. Bull. Johns Hopkins Hosp., xxxiv, 316.

BOLLMAN, J. L., C. SHEARD AND F. C. MANN. 1927. This Journal, lxxx, 461.

FLEISCHL, E. 1874. Ber. u. d. Verhandl. d. K. Säch. Gesell. d. Wissench. z. Liepzig, xxvi, 42.

Gerhardt, D. 1897. Verhandl. d. Kong. f. inn. Med., xv, 460.
Greene, C. H., Snell, A. M. and Walters, Waltman: Diseases of the liver. I. A survey of tests for hepatic function. Arch. Int. Med., 1925, xxxvi, 248–272.
Kufferath. 1880. Arch. f. Physiol., 92.
Kunkel, A. 1875. Ber. u. d. Verhandl. d. K. Säch. Gesell. d. Wissench. z. Leipzig, xxvii, 232.
McIndoe, A. H. Personal communication.
Mall, F. P. 1906. Amer. Journ. Anat., v, 227.
Mendel, L. B. and F. P. Underhill. 1905. This Journal, xiv, 252.
Merkel, F. 1915. Die Anatomie des Menschen, mit hinweisen auf die artzliche praxis. Wiesbaden, J. F. Bergmann, Pt. IV, Fig. 155, p. 57.
Minkowski, O. 1904. Zeitschr. f. klin. Med., iv, 34.
Saunders, W. 1803. A treatise on the structure, economy, and diseases of the liver; together with an inquiry into the properties and component parts of the bile and biliary concretions. Ed. 3, London, W. Phillips, 111–115.
Snell, A. M., C. H. Greene and L. G. Rowntree. 1927. Arch. Int. Med., xl, 471.
Tiedemann, F. and L. Gmelin. 1827. Die Verdauung nach Versuchen. Leipzig, K. Gross, ii, p. 40.
Walters, W. and J. L. Bollman. 1928. Jour. Am. Med. Assn., xxi, 239.
Wertheimer, E. and L. Lepage. 1897. Arch. de physiol. norm. et path., Ser. 5, ix, 363.
1898. Arch. de physiol. norm. et path., Ser. 5, x, 334.
1899. Journ. de physiol. et de path. gén., i, 259.
Whipple, G. H. and J. H. King. 1911. Journ. Exper. Med., xiii, 115.
Whipple, G. H. 1922. Physiol. Rev., ii, 440.

NERVOUS CONTROL OF RESPIRATION

I. Observations on the Localization of the Respiratory Mechanism in the Isthmus, Pons and Upper Medulla of the Cat

ALLEN D. KELLER

From the Physiological Laboratory, Cornell Medical College, Ithaca, N. Y.

Received for publication April 5, 1929

A. Operative procedure and methods. Cats were used and anesthetized with approximately one gram of urethane per kilogram of body weight administered with a stomach tube. Deep anesthesia (surgical) was maintained with ether during surgical procedures. A tracheal cannula was introduced and carotids were ligated. Blood pressure was taken from a femoral or carotid artery. Respiratory records were taken by means of a Paul Bert pneumograph or by introducing a balloon into the abdominal cavity. The superior colliculi were exposed from above by removal of the occipital poles of the cerebral hemispheres. The floor of the fourth ventricle was exposed by means of a caudal approach as follows: The occipital muscles were spread apart along the dorsal mid-line and held apart by retractors. The occipital bone was exposed and a small piece dorsal to and adjoining the magnum foramen was snipped out with bone forceps. The removal of this small area of bone exposed the ventrocaudal aspect of the cerebellar vermis which was then removed with a small spoon. Hemorrhage was controlled with small wads of cotton. The removal of the cerebellar vermis exposed the floor of the fourth ventricle, usually with a minimum of shock to the respiratory and vasomotor mechanisms. (See A, plate III.) Lesions were made in the stem (ventral to floor) by means of knives especially designed for particular lesions.

Polypnea occurred in some of the preparations spontaneously as they came from under surgical anesthesia (ether removed). In other preparations it was induced by a moderately strong stimulation of a central sciatic stump. (See tracing summary cat 47.) In preparations in which polypnea did not occur in these ways it was induced by playing a heat beam upon the animal's body by means of an ordinary 600 watt electric heater. The intactness of the polypnea mechanism following lesions was also tested in this manner.

At the close of the experiment the brain and brain-stem were removed and hardened in 10 per cent formalin for 24 hours. The cerebellum was removed and the location of the lesion in the floor of the fourth ventricle

was carefully noted and recorded. The stem was then sectioned into suitable blocks and placed in 3 per cent aqueous sodium dichromate solution. After 2 to 4 weeks in dichromate, the tissue was embedded in celloidin and sectioned. At the time of sectioning, the location of lesions was carefully noted and charted on photographic prints of transverse sections of the stem at various levels. (See lesion charts in plates I and II.)

B. Polypnea mechanism. Richet (1898) designated two types of polypnea, reflex and central, according to the mode of genesis. Polypnea occurring in a normal dog exposed to the sun's rays was concluded by him to be reflex in origin since polypnea developed without a rise in rectal temperature. He then demonstrated that animals under a surgical depth of anesthesia did not develop polypnea until a rectal temperature of 41.7°C. was reached. He concluded, therefore, that polypnea occurring under these conditions is central in origin since afferent impulses would supposedly be eliminated by a surgical depth of anesthesia.

Nikolaides and Dontas (1911) reported that central heat polypnea *could not be induced in the dog unless the corpora striatum was intact.* Bazett and Penfield (1922) observed in a cat decerebrated through the middle of the corpora quadrigemina (chronic experiment) a rectal temperature of 42.8°C. and at the same time a respiratory rate of only 60 per minute. They further stated that cats with the stem hemi-sectioned at the level of the corpora quadrigemina responded in a more marked degree to heat than did the cats in which the stem was completely sectioned. They gave no figures, however.

C. S. Sherrington (1924) tested the respiratory response to heat of a

---

Plate I. Lesion Charts

Cat 23. *C.M.L.*, cephalic mid-line lesion; *R.L.L.*, right lateral lesion; *L.L.L.*, left lateral lesion; *C.T.L.*, caudal transverse lesion.

Cat 27. *C.T.L.*, mid-line caudal lesion; *L.L.L.*, left lateral lesion; *R.L.L.*, right lateral lesion.

Cat 28. *L.R.L.*, left reticular lesion; *R.R.L.*, right reticular lesion; *C.T.L.*, caudal transverse lesion; *L.L.L.*, left lateral lesion; *R.L.L.*, right lateral lesion.

Cat 50. *R.L.L.*, right lateral lesion; *L.L.L.*, left lateral lesion.

Cat 51. *L.M.L.*, left medio-lateral lesion; *R.M.L.*, right medio-lateral lesion.

Cat 60. *Lesion*, medial transverse; *L.L.L.*, left lateral lesion.

Plate II. Lesion Charts

Cat 30. *C.L.*, cephalic mid-line and transverse lesions; *L.R.L.*, left reticular lesion; *R.R.L.*, right reticular lesion; *C.C.L.*, cephalic collicular lesion; *R.L.L.*, right lateral lesion.

Cat 46. *T.L.*, transverse lesion.

Cat 49. *C.L.*, caudal transverse lesion; *R.L.L.*, right lateral lesion; *L.L.L.*, left lateral lesion.

Cat 53. *R.C.C.*, right cephalic cut; *L.C.C.*, left cephalic cut; *C.C.*, caudal cut.

Cat 55. *M.T.L.*, medial transverse lesion; *L.L.L.*, left lateral lesion; *R.L.L.*, right lateral lesion.

Cat 62. *Lesion*, transverse lesion.

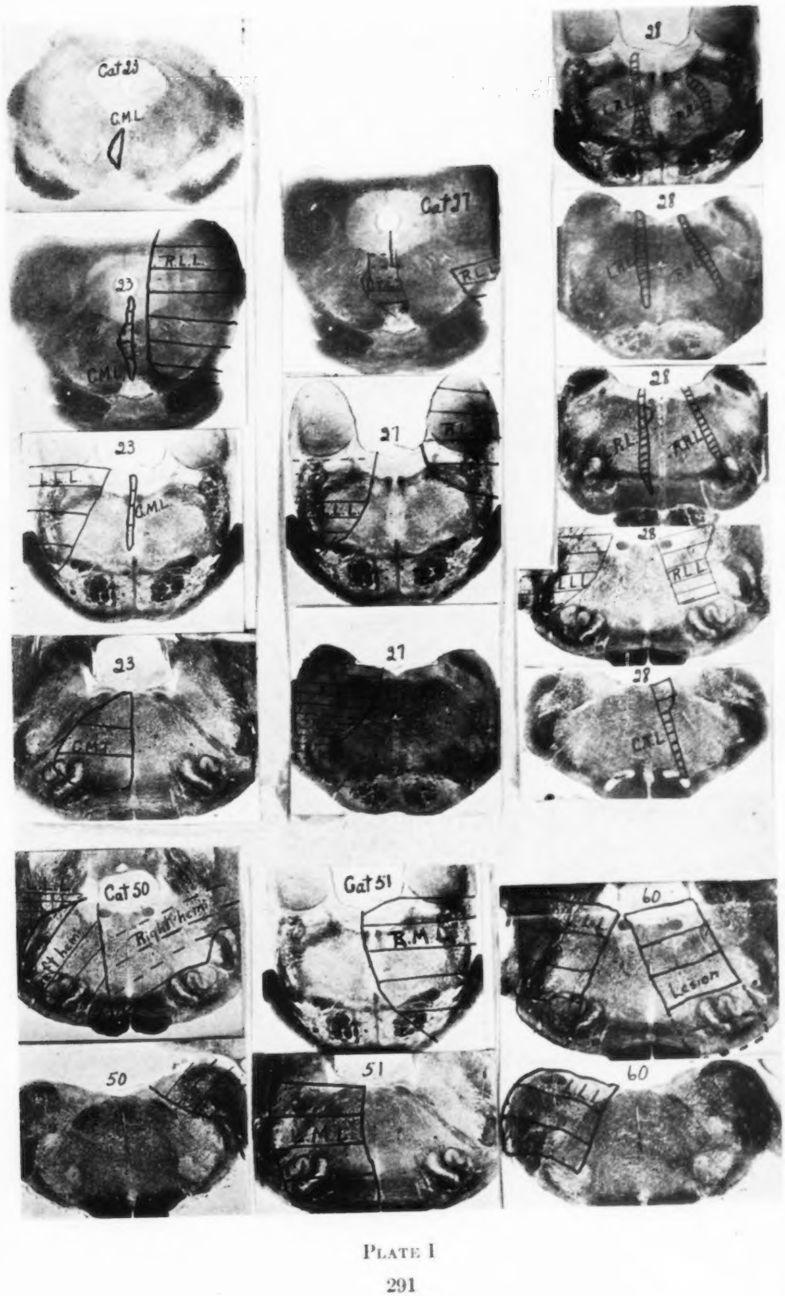

Cat 23
C.M.L.
23
R.L.L.
C.M.L.
23
L.L.L.
C.M.L.
23
C.M.T.
Cat 27
R.L.L.
27
R.L.L.
L.L.L.
27
28
L.R.L.
R.R.L.
28
28
R.R.L.
28
L.L.L.
R.L.L.
28
C.R.L.
Cat 50
Left hemi
Right hemi
50
Cat 51
R.M.L.
51
L.M.T.
60
Lesion
60
L.L.L.

PLATE I

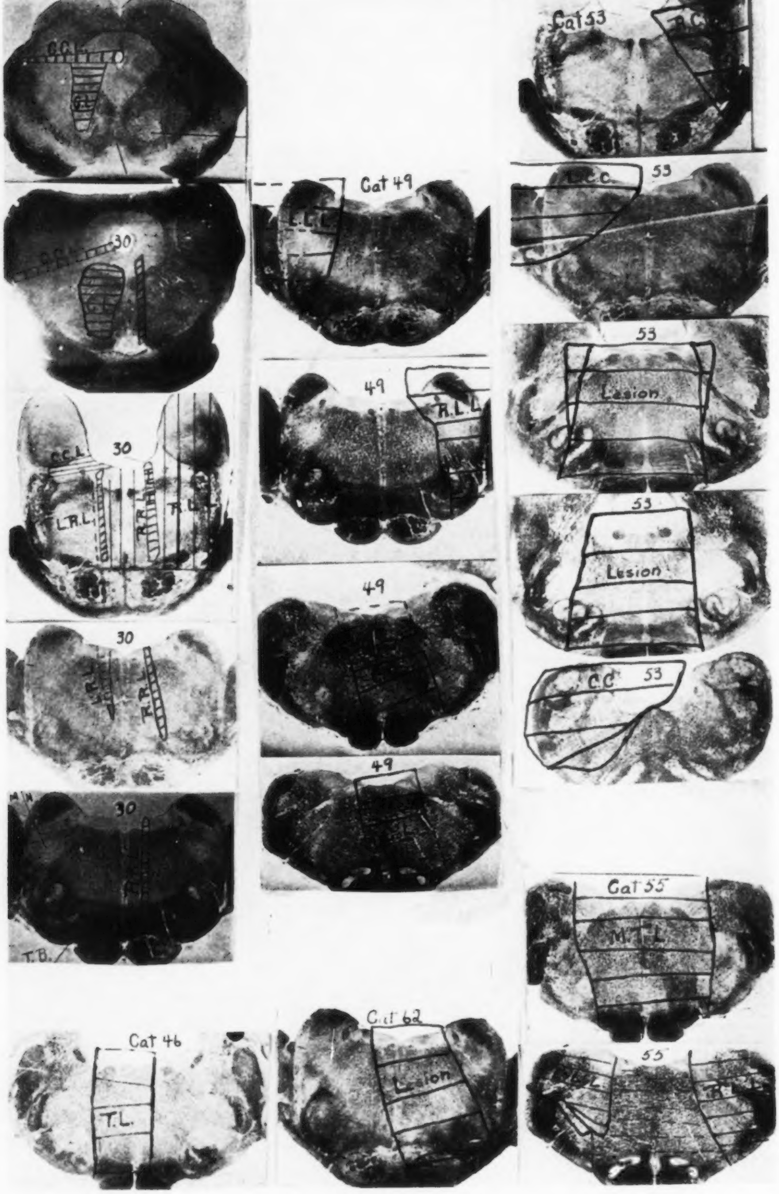

C.C.L.
30
C.C.L.
30
L.R.L.
R.R.L.
30
L.R.L.
R.R.L.
30
T.B.
Cat 49
L.C.L.
49
R.L.L.
49
49
Cat 53
53
53
Lesion
53
Lesion
C.C
53
Cat 55
M.T.L.
55
Cat 46
T.L.
Cat 62
Lesion

PLATE II

cat decerebrated 4 mm. anterior to the inferior colliculi (acute experiment). In this case a rise in rectal temperature was accompanied by an increase in respiratory rate. Typical polypnea, however, did not occur. His figures are quoted below.

RECTAL TEMPERATURE	RESPIRATORY RATE
Before heating	30
41.1°C.	75
42.5°C.	66
42.7°C.	Succumbed to heat exposure

As a result of these observations it is assumed by the above investigators that the heat polypnea center is located somewhere in the cephalic stem. If reflex polypnea then is dependent upon cephalically situated centers, one might expect that the course taken by the afferent tracts going to and efferent tracts coming from the centers might be localized in definite regions in the upper medulla and pons. Such a localization was attempted and the procedure and results given in this paper.

1. Origin of light anesthesia polypnea. Garrelon and Langlois (1907), Henderson (1910), and Nikolaides and Dontas (1911) report cases of polypnea occurring in experimental animals under light anesthesia. Nikolaides and Dontas describe polypnea occurring spontaneously in a dog lightly narcotized by morphine in a room temperature of 17.0°C. These observers agree that polypnea occurring under these conditions is reflex and not central in origin since the rectal temperature is not increased.

By using an intermediate depth of anesthesia, that is, deep enough to prevent polypnea developing spontaneously and at the same time not so deep as to necessitate a marked raising of the rectal temperature in order to induce polypnea, the reflex origin of light anesthesia polypnea was demonstrated, as shown by the following example:

Cat 59

TIME	RECTAL TEMPERATURE	RESPIRATORY RATE
	°C.	
10:04	35.2	39
Heat on		
10:06	35.5	66
10:08	35.8	72
10:10	36.0	90
Heat off		132
10:12		72
10:14	36.8	54
10:20	37.1	42
10:32	37.2	39

These figures show that the heat beam produced a rise in rectal temperature as well as an increase in the respiratory rate. Following the removal of the heat beam the respiratory rate gradually returned to its original rate whereas the rectal temperature continued to rise. In a great number of cases polypnea was induced by the heat beam before the rectal temperature began to rise. The acceleration of the respiratory rate in these cases is therefore caused by afferent stimulation set up by the heat beam on the skin and not by the increased temperature of the blood passing over the polypnea centers in the brain-stem. So, we were clearly dealing with afferent conduction to the polypnea centers as well as efferent conduction from these centers.

2. The sensitivity of reflex polypnea to surgical manipulation. Early in this investigation it was recognized that light anesthesia polypnea could be eliminated, presumably by shock, in the process of exposing the corpora quadrigemina from above (removal of occipital poles), that is, without placing a lesion in the stem.

Example: Cat 39. The floor of the fourth ventricle was exposed before records were taken.

RECTAL TEMPERATURE	RESPIRATORY RATE
°C.	
38.0	190 (spontaneous development of polypnea)
Heat on	
38.0	210
Lesion	210–168
38.0	198
38.6	246
Heat off	
Interval, during which the corpora quadrigemina was exposed by removal of the occipital poles from above. Rather profuse hemorrhage occurred.	
40.0 Under some ether	36
39.5 Free from ether	66
Heat on	
40.0	75
Heat off	

In this cat the depth of urethane anesthesia was such that after the floor of the fourth ventricle was exposed polypnea developed spontaneously as the animal came out from under the influence of ether. The heat beam increased polypnea still further which was still intact after placing lesions in the medial stem. It was eliminated, however, during the interval in which the colliculi were exposed, since it did not again occur spontaneously as the animal came from under ether. Although the heat beam acceler-

ated the respiratory rate it did not elicit polypnea. After polypnea was eliminated the ordinary quiet respiratory mechanism was still intact.

On the other hand, it is to be emphasized that the corpora quadrigemina can be exposed by removal of the occipital poles without interfering with polypnea in any way as is shown by cat 17.

Cat 17. The corpora quadrigemina were exposed before polypnea was elicited.

TIME	RECTAL TEMPERATURE	RESPIRATORY RATE
	°C.	
11:15	37.0	66
	Heat on	
11:18	37.0	132
Cephalic transection of the stem just in front of the superior colliculi		126–126–126
	Heat off	
11:22		110
After interval during which sciatic reflexes were tested.		72
	Heat on	
Transverse section of the stem through middle of superior colliculi		114–apnea–150
	Heat off	
At this point the bony tentorium was removed with the idea of sectioning the stem at a lower level, e.g., caudal to the inferior colliculi. After above interval.		50
12:15		36
	Heat on	
12:19	*Heat off*—animal died	19

It is to be noted in this cat that not only were the colliculi exposed but also the stem was completely sectioned as far caudad as the middle level of the superior colliculi without eliminating polypnea. Polypnea, however, was eliminated by manipulation in removing the bony tentorium. Of special note is the fact that the heat beam induced polypnea before shock set in, whereas after shock it slowed the respiratory rate and caused the death of the animal. Careful post-removal examination of the stem revealed no injury to the stem by such manipulation. It might be stated here that in three attempts to expose the floor of the fourth ventricle by removal of the tentorium from above and two attempts to expose the region caudal to inferior colliculi by removal of anterior portion of the cerebellum from above, polypnea was eliminated and could not be induced again. Thus, the area about the caudal end of the inferior colliculi seems especially sensitive (see also left lesion, cat 30).

Polypnea can also be eliminated in exposing the floor of the fourth ventricle by means of the caudal approach. In cat 24, for instance, in removing the cerebellar vermis the respiratory rate was slowed from 222 per

minute to 60 per minute. A very slight dip of the spoon into the left side of the stem at the caudal level of the inferior colliculi was revealed when a post-removal examination of the stem was made.

The above experimental observation demonstrates conclusively that light anesthesia polypnea can be interfered with and totally abolished, at least temporarily, without placing a lesion in the stem. It can, therefore, be said that *if a lesion interferes with polypnea, it is not conclusive proof that the lesion has destroyed any tracts or centers concerned with the polypnea mechanism.*

Since the stem can be sectioned as far caudad as the middle level of the inferior colliculi without eliminating polypnea (cat 17) it would suggest that Nikolaides and Dontas' results were cases of shock to the polypnea mechanism rather than destruction of anatomical structures concerned with the mechanism.

3. Localization of the reflex polypnea mechanism in the isthmus, pons and upper medulla. a. *Protocols.* The respiratory rates and amplitudes given in the tracing summaries and elsewhere were read from graphic tracings (not shown).

Cat 23. Tracing summary

TIME	RECTAL TEMPERATURE	LESION	RESPIRATORY RATE
	°C.		
	38.2		
		C.M.L.*	180 –138
	37.9	C.T.L.	168–174–126
		L.L.L.*	126– 28– 48
	37.9	R.L.L.	48– 24– 33
10:47		R.V.C.	33– 23– 19
10:50		L.V.C.	20– 17– 10–18
11:08	38.0		18
		Heat on	
11:15	38.7		20
11:20	40.0		26
11:24	41.0		25
11:25	42.0		25
11:30	42.9		30
Later			21–gasping followed

* See A, plate III.

Cat 23 was allowed to come from under ether before exposing the floor of the fourth ventricle. Polypnea developed spontaneously. Polypnea was not interfered with during the exposure of the floor. The effect of the cephalic mid-line lesion was a slight temporary slowing of the respiratory rate (see A, plate III). This lesion was a narrow medial longitudinal slit that severed all fibers crossing the mid-line from the cephalic level of the red nuclei to the middle level of the pons.

Following the caudal transverse lesion the respiratory record was somewhat

irregular. Polypnea was partially eliminated. Thus the tracing shows in addition to the usual long respiratory excursions that are characteristic of ordinary quiet breathing, also shorter rapid excursions which are superimposed on the inspiratory phase of the longer respiratory excursions. This lesion involved the region medial to the exiting facial root and lateral to the mid-line of the left side at the level of the

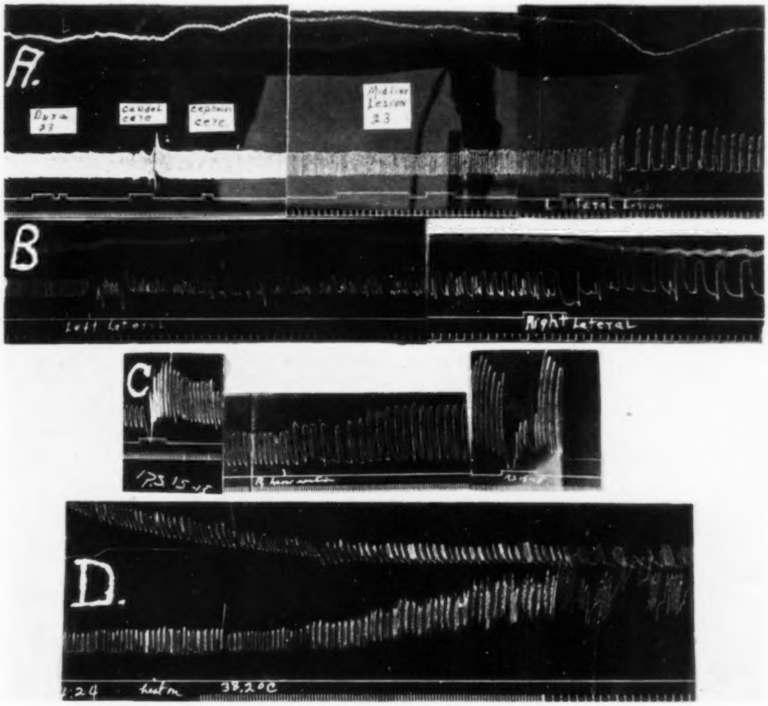


PLATE III. TIME, 1 PER SECOND

A, Cat 23, insp., downstroke. B, Cat 27, insp., downstroke. C, Cat 50, insp., downstroke. D, Cat 60, insp., upstroke.

outgoing roots of the facial nerves. The extreme ventral portion of the stem was left intact.

The left lateral lesion eliminated the rapid respiration characteristic of polypnea (see A, plate III). After this lesion, respiration was characteristic in rate and depth of respiration of an animal under a surgical depth of anesthesia. The lesion cut the extreme lateral stem transversely at the middle level of the pons.

The right lateral lesion slowed the respiratory rate still more and the amplitude of the respiratory excursions increased. This response is typical of that which occurs in an animal under a surgical depth of anaesthesia, when the complete stem is sectioned through or caudal to the inferior colliculi.

Following this lesion the vagi were cut and respiration became slower.

The preparation then responded to the heat beam by a rise in rectal temperature and an increase of the respiratory rate. However, the respiratory response was very slight. Since the animal succumbed to heat at a rectal temperature of 43.0°C., these lesions eliminated the central polypnea mechanism as well as the reflex polypnea mechanism.

Cat 27. Tracing summary

RECTAL TEMPERATURE	LESION	RESPIRATORY RATE
°C.		
36.0		258
	Mid-line cephalic	220-222-222
	Mid-line caudal	222-222-222
	Left lateral *	240-short excursions
36.0	Right lateral *	short excursions-24
	R. V. cut	21- 10
36.1	L. V. cut	10-insp. apnea-gasping

* See B, plate III.

The floor of the fourth ventricle was exposed before records were taken. Polypnea occurred spontaneously.

The mid-line cephalic lesion had no effect on respiration. This lesion was a very narrow slit involving only the central grey matter ventral to the cerebral aqueduct at the level of the cephalic pons. It is represented in lesion chart by a narrow black line.

The mid-line caudal lesion was without effect. This lesion was a limited transverse slit through the medial stems at the cephalic level of the pons.

The left lateral lesion altered the respiratory rate markedly. On the tracing the two types of excursions noted in the case of cat 23 can be seen (see B, plate III). The larger excursions have a prolonged inactive expiratory phase while short rapid excursions are superimposed upon the inspiratory phase of the former. The left lateral lesion involved the lateral portion of the left stem. The cut entered the stem at the level just cephalad to the masticator nuclei and passed ventrally in a cephalic direction so that the ventro-cephalic limit of the cut was at the middle level of the pons.

The right lateral lesion eliminated the short rapid excursions entirely. The respiratory movements following this lesion were characteristic of a surgical depth of anesthesia. The lesion involved the dorso-lateral portion of the right stem at the middle level of the pons.

Cat 28. Tracing summary

In this cat the left reticular lesion, the right reticular lesion and the caudal transverse lesion were placed before polypnea was induced. Aside from a temporary apnea the respiratory movements were not altered by these lesions. The left reticular lesion was a narrow longitudinal slit involving the medial reticular formation as charted from the extreme cephalic level of the exiting facial roots to practically the cephalic level of the pons. The right reticular lesion involved the lateral reticular formation as charted. This lesion did not reach as far caudad or cephalad as the left lesion. The caudal transverse lesion was a narrow slit that involved the

reticular formation as charted. This lesion occurred at a slightly higher level than that on which it is charted.

TIME	RECTAL TEMPERATURE	LESION	RESPIRATORY RATE
	°C.		
	38.2		21
		Left reticular	33–apnea–33
		Right reticular	30–18–30–18
		Caudal transverse	27–apnea–27
11:15			24
		Heat on	
11:35	40.0		180
	40.0	Left lateral	180–60–short excursions
		Heat off	
		Left vagus cut	192–48–204
	40.5	Right vagus cut	192–48–48
	40.5	Right lateral	162–48
Later			27
		Heat on	
12:15	40.7		48
12:30	40.8		120–short excursions
		Heat off	
12:45	39.5		42
1:00	39.0		30
1:15	38.2		20
3:40	36.0		13
The animal was warmed up here gradually by warming table			
8:30	40.6		21
		Heat on	
8:35	40.6		24–short excursions
8:50	42.0		36–increased number of short excursions
8:55	43.0		gasping

After the above lesions were placed, polypnea was readily induced by means of the heat beam. As a result of the left lateral lesion the respiratory rate was characteristically and temporarily slowed. It is to be noted that the short rapid excursions soon appeared. This lesion cut the extreme lateral stem on the left side as charted at the level of the facial genui.

The right lateral lesion eliminated the short excursions. It involved the region of the right stem dorsal to the superior olive, a portion of the trigeminal nucleus being involved.

The following points should be noted on the above tracing: Although the left lateral lesion involved the fifth spinal root and nucleus, polypnea was only slightly affected: In spite of the lesions and the absence of the vagi the animal continued to respire very nicely and adequately for nine hours. At that time it succumbed because of over-heating: The polypnea mechanism was not any further recovered at 8:50 p.m. than it was at 12:30 noon: The heat beam increased the rate both of the ordinary respiratory excursions as well as the short rapid excursions: Stimulation of the central sciatic stump with moderately strong shocks eliminated polypnea during the time of stimulation.

Cat 30. Tracing summary

Lesions	Respiratory rate
Cephalic mid-line	240–150–220
Caudal mid-line	200–170
Cephalic transverse	200–200–200
Second transverse	225–170–200
Third transverse	240–230–240
Left reticular	250–250–250
Right reticular	240–240–240
Cephalic collicular	260– 60– 80
Right lateral	80– 22– 36

The cephalic mid-line and transverse lesions merged in one another. However, they involved the left medial stem of the isthmus and cephalic pons. This lesion was very extensive, more so than charted, and the space was filled with a huge blood clot. The tracing summary shows that polypnea was still intact following these lesions. The left reticular lesion was a narrow longitudinal slit involving the medial reticular formation of the pons. This lesion did not affect polypnea in any way.

The right reticular lesion involved the medial reticular formation of isthmus, pons and upper medulla, reaching as far caudad as the exiting roots of the facial nerves. This lesion had no affect upon polypnea.

The cephalic collicular lesion was made by pushing the spoon forward through the collicular bodies on the left side. It practically separated the collicular bodies from the stem. This lesion immediately eliminated typical polypnea.

The right lateral lesion sectioned the medial portion of the left stem and the complete right stem at the middle level of the pons. This lesion markedly slowed the respiratory rate.

The above lesions demonstrate that in the same animal although extensive lesions of the medial stems did not affect polypnea yet a lesion involving the extreme dorsolateral portion of the stem eliminated polypnea immediately. This experiment also shows that the region caudal to the inferior colliculi is especially sensitive (see preceding discussion).

Cat 46. The transverse lesion involving all the medial part of the left stem, cutting the left medial longitudinal bundle completely and the medial portion of the right bundle, did not affect polypnea in any way. The respiratory rate of 190 per minute was still maintained following the lesion. This lesion did not extend far into the lateral stem so that the lateral reticular formation was intact. The cut was at the cephalic level of the facial nuclei.

Cat 47. The lesion in cat 47 involved practically the same part of the stem as in cat 46 except that it was located more caudad, e.g., at the middle or caudal level of the facial nuclei. This lesion did not alter the respiratory rate except that the amplitude was decreased temporarily. This temporary decrease in amplitude occurred in all caudally placed lesions. Following this lesion polypnea was still induced by stimulating the central sciatic stump. Thus, before the lesion R. S. 17–15 increased the respiratory rate from 42 per minute to 90 per minute, this accelerated rate being maintained for some time following stimulation. After the lesion R. S. 17–15 increased the respiratory rate from 48 per minute to 102 per minute, the accelerated rate being maintained for some time following stimulation.

Cat 49. Tracing summary

TIME	RECTAL TEMPERATURE	LESION	RESPIRATORY RATE
	°C.		
First record			108
		Left vagus cut	162-150-140
	38.0	Right vagus cut	180- 84- 50
Interval during which sciatics were stimulated			
4:43	37.0	Caudal T.	200-apnea-145-132
Another interval, sciatics stimulated			
		Right lateral	192- 60- 84
		Left lateral	30-insp. apnea

Cutting the first vagus did not markedly alter the respiratory rate. Cutting the second vagus eliminated polypnea temporarily. However, at the time of placing the caudal transverse lesion polypnea was again present, the respiratory rate being above any previous rate.

The caudal lesion slowed the respiratory rate. However, recovery occurred as typical polypnea was present at the time of placing the right lateral lesion. The caudal lesion cut completely both medial longitudinal bundles at the extreme caudal level of the facial nuclei. The lesion extended further lateralad on the right side.

The right lateral lesion cut the right lateral stem at the level of the masticator nucleus. This lesion eliminated polypnea. Respiratory movements were, however, still present.

The left lateral lesion cut the left lateral stem at the level just cephalad to the superior olive and the masticator nucleus. This lesion eliminated all respiratory movements.

Cat 50. Tracing summary

TIME	RECTAL TEMPERATURE	LESION	RESPIRATORY RATE
	°C.		
	38.2	R.S. 13-15	30-156- 84
		R. lateral	288- 60
		L. lateral	312-240-156
		L. hemi-section	360- 30- 96
11:35	38.2		27
		R.S. 15-15	14-114- 54-18
11:45	38.5		14
		Heat on	
11:49	39.0	(Marked increase in amplitude and presence of short excur-	21
11:54	40.0	sions on inspiratory phase)	27
		Heat off	
		(Increased amplitude and short excursions disappeared at once)	
12:07	41.0		39
		R.S. 15-15 *	28-132- 36
12:20	40.5	R. hemi-section *	28- 16- 16
		R.S. 15-15 *	16-16-apnea-16-apnea-16

* See C, plate III.

Died shortly after last stimulation from respiratory failure.

In this cat the sciatic reflexes were tested before and after the lesions. Before polypnea was induced R.S. 13–15 produced a marked accelerated respiration response. Following the right lateral lesion the short excursions of polypnea gave way to the slower and higher excursions characterized by ordinary breathing. However, polypnea soon appeared again. The right lateral lesion involved the extreme dorsolateral region of the stem at the level of the facial nuclei leaving the trigeminal root and nucleus intact.

The left lateral lesion affected polypnea slightly but only temporarily. The lesion cut the extreme lateral stem at the level of the facial genu, involving only the vesticular nuclei and cerebellar peduncles.

The left hemi-section eliminated polypnea. It cut the left stem with the exception of the extreme medial portion at the level of the facial genu. Following these lesions the "respiratory acceleration reflex" elicited through the right sciatic was still intact (see C. plate III).

The right hemi-section slowed the respiratory rate further. This slowing is of the same character as when the complete brain stem is severed caudal to the corpora quadrigemina under a surgical depth of anesthesia. The right hemi-section cut the complete right stem with the exception of a narrow ventro-lateral strip. Thus, a portion of the trigeminal root was left intact. Following the right hemi-section the accelerated reflex response through the sciatic was eliminated (see C, plate. III) The respiratory responses induced through a sciatic after the left hemi-section differed from that obtained before in that a short period of apnea ensued before the respiratory rate was accelerated. This period of apnea still appeared following the right hemi-section.

Cat 51. Tracing summary

TIME	RECTAL TEMPERATURE	LESION	RESPIRATORY RATE
	°C.		
12:10		Left lateral	104–132–72
12:11			48
		R.S. 13–15	84–138
		R.S. 15–15	54–138
	34.5	*Heat on*	48
	35.0		Short excursions
		Heat off	
		R. lateral	40– 48–21
	36.0		18
		R.S. 13–15	18–apnea–18
		R.S. 15–15	18–apnea–30
12:44		L.V. cut	12–apnea– 4
12:51		L.V. cut	3–insp. apnea–gasping

The left medial lateral lesion did not eliminate polypnea at once when the lesion was placed. However, shortly after the making of the lesion polypnea was gradually replaced by a slower respiratory rate. This lesion sectioned the complete left stem at the level of the exiting facial roots with the possible exception of an extreme lateral strip, thus leaving a portion of the trigeminal root intact. Following this lesion the rapid acceleration reflex was elicited through the right sciatic.

The right medial lateral lesion slowed the respiratory rate further and eliminated

the rapid acceleration reflex. This lesion cut the right stem completely at the middle level of the pons.

Cat 53. Tracing summary

TIME	RECTAL TEMPERATURE	LESION	RESPIRATORY RATE
	°C.		
		Floor exposed	
4:40	36.1		39
4:51		R.S. 17–15	47–102–96
5:04	36.5	*Heat on*	42
5:16	38.0	Lesion	120–apnea– 70
		Heat off	
5:20	38.5	R.S. 17–15	144–126–156
		R.S. 17–15	156–168–168
		R. cep. cut	200–156–108
		L. cep. cut	108–120– 78
		R.S. 17–15	78– 54– 66
		Caudal cut	66–apnea–33

The lesion slowed the respiratory rate temporarily. However, polypnea was present four minutes after the lesion was placed. The respiratory acceleration reflex was still intact. The lesion completely severed the medial stems at the level of the exiting facial roots. The lesion extended further lateral on the left side than on the right side.

The right cephalic cut and left cephalic cut were placed, one shortly after the other. They did not eliminate polypnea immediately. However, a gradual slowing of the respiratory rate occurred following the lesions. The "respiratory acceleration reflex" response was not elicited through the sciatic following the above lesions. The right cephalic cut sectioned the right lateral stem at the middle level of the pons. The left cephalic cut sectioned the dorsal portion of the left stem at the caudal level of the pons.

The caudal cut markedly slowed the respiratory rate. The caudal cut involved the stem at the level of the obex as charted.

Cat 55. Tracing summary

TIME	RECTAL TEMPERATURE	LESION	RESPIRATORY RATE
	°C.		
12:47		M. transverse	300–expiratory apnea
1:00			gasping
1:10			48
1:14	39.5		60
2:18	40.0		240
	40.3	R.S. 13–15	216–*rapid*–*156*–216
2:57		L. lateral	216–apnea–240–246
3:01	40.5		120
3:55	41.0	R.S. 13–15	114–*rapid*– *90*– 84
3:59		*Heat on*	120
4:00			96
4:02	41.0		90
		Heat off	
4:04			120
		R. lateral	150–gasping–death

The medial transverse lesion produced complete apnea which was followed by dyspnea. The dyspnea was gradually displaced by normal breathing. Records were not taken from 1:10 until 2:18. At 2:18 typical polypnea had again returned. The lesion completely sectioned the medial stems at the middle level of the facial nuclei.

The respiratory rate was gradually slowed after placing the left lateral lesion. However, coördinated and fairly rapid respiration continued following this lesion. After this lesion the heat beam produced a slowing of the respiratory rate. The left lateral lesion cut the dorso-lateral stem at the caudal level of the facial nuclei.

The right lateral lesion was followed by gasping. It cut the right lateral stem at the caudal level of the facial nuclei.

Cat 60. Tracing summary

TIME	RECTAL TEMPERATURE	LESION	RESPIRATORY RATE
	°C.		
11:38	35.0	Medial transverse	180–apnea—irregular
11:40			54–short excursions
11:46	35.0		150
11:50	35.0		114–162
11:57	35.0	*Heat on*	108
11:59	35.0		132
12:00		*Heat off*	174
12:01		Left lateral	150–apnea–24
12:03	35.1		24
12:12		R.S. 13–15	25– 48–42
12:17		R.S. 17–15	18–24–36
12:20	36.0	*Heat on*	24
12:21			21
12:22		*Heat off*	18–26
4:20	38.2	R.S. 17–15	72–short excursions
4:24	38.2	*Heat on**	48–short excursions
4:27	38.5	*Heat off*	
4:38			42
4:41		L.S. 17–15	54–short excursions–66

*See D, plate III.

The medial transverse lesion produced a temporary apnea which was followed by more or less uncoördinated respiratory movements. However, complete recovery soon occurred. The rapid acceleration reflex was intact and the animal responded to the heat beam by an acceleration in the respiratory rate. The lesion involved the right stem medial to the existing root of the facial nerve. It is to be noted that this lesion would involve the spino-thalamic tract on the right side.

The left lateral lesion eliminated polypnea. The rapid acceleration reflex from the sciatic was not intact immediately following the lesion. However, four hours later this reflex was partly present as shown by the presence of short rapid excursions superimposed upon the inspiratory phase of the slower excursions. An hour later the reflex was still further recovered as shown by the presence of more short excursions during stimulation of the sciatic. Immediately following the left lateral lesion the animal responded to the heat beam by a decrease in the respiratory rate whereas four and one-half hours later it responded by the appearance of rapid excursions as described above. (See D, plate III.)

Cat 62. Tracing summary

TIME	RECTAL TEMPERATURE	LESION	RESPIRATORY RATE
	°C.		
11:28	36.8		42
		Heat on	
11:44	38.0		96
		Heat off	
	40.5		300
12:09	40.8	Transverse	288-276-252
12:28	40.0	R.S. 13-15	180-234-216
12:33	40.0	*Heat on*	120
12:35	40.0		156
12:37	40.0		204
12:41	40.5	*Heat off*	204
12:45			180

The lesion in this cat cut the right stem completely except the dorso-lateral portion. The lesion did not markedly slow the respiratory rate. The apparent slowing as shown by the tracing summary was due to the already beginning decline as a result of cessation of the action of the heat beam. The preparation responded to the heat beam following the lesion with a marked increase in the respiratory rate.

b. *Discussion.* The foregoing experiments show that extensive lesions can be placed in the medial stems of the upper medulla and pons without interfering with reflex polypnea. The cephalic midline lesion in cat 23, cephalic lesions in cat 27, cephalic lesions in cat 30, reticular lesions in cats 28 and 30, the medial transverse lesions in cats 46 and 47, and the transverse lesion in cat 62 affected polypnea in no appreciable way. The medial transverse lesion in cat 23, medial lesion in cat 49, medial lesion in cat 53, and medial lesion in cat 55 affected polypnea only temporarily since complete recovery of the polypnea mechanism occurred in all of these cases.

The transection of the brain-stem in cat 17 through the middle level of the superior colliculi without eliminating polypnea would locate the reflex polypnea centers caudal to this level in the brain-stem. The cephalic midline lesion in cat 23 eliminates the possibility of the reflex polypnea centers being dependent on fiber tracts crossing the midline at the isthmus or pons level. The cephalic midline and cephalic transverse lesions in cat 30, reticular lesions in cats 30 and 28, and especially the transverse lesion in cat 62 would eliminate the possibility of the polypnea centers being located in the medial stems (reticular formation) of the cephalic pons or isthmus. Thus, if polypnea is dependent on fiber tracts or "centers" located in the pons or isthmus, they are confined to the extreme lateral regions of the stems.

The transverse lesions in cats 53 and 55 also eliminate the possibility of these centers being dependent on fiber tracts passing cephalad or caudad

through the medial stems of the upper medulla. Reflex polypnea is, therefore, not dependent on the reticulo-spinal tracts that take origin from the reticular nuclei of the upper medulla, pons and isthmus (Papez, 1926) since these lesions would have severed these tracts. It has been suggested by Allen (1927) that these tracts are respiratory in function.

These results show that if reflex polypnea is dependent on anatomical centers located in the brain-stem cephalad to the facial nuclei, the afferent tracts supplying these centers and the efferent tracts leading from them course through the lateral regions of the upper medulla and pons in close proximity to the trigeminal roots and nuclei and the spino-thalamic tracts.

That reflex polypnea is dependent on fiber tracts which pass through the upper medulla and pons is suggested by the fact, as seen from tracings, that all lateral lesions invariably interfered with polypnea. However, the effects of similar lateral lesions upon the reflex polypnea mechanism are somewhat variable. For instance, the left lateral lesions in cats 23, 27 and 53 are practically identical in location and yet they all affected polypnea in a somewhat different manner. In cat 23 polypnea was immediately and completely eliminated. In cat 27 polypnea was altered immediately but was only partially eliminated as shown by the fact that short rapid respiratory excursions continued to appear at intervals. In cat 53 polypnea was not immediately altered by the lateral lesions but the respiratory rate became gradually slower some time after the lesion was placed.

The factors involved in producing such variability of results by similar lesions can not be fully determined from the experimental data obtained thus far.

In regard to the short excursions which are very definitely associated with a unilateral lesion, the question naturally arises "Is such a partial elimination of polypnea due to a cutting of tracts leading to and from the polypnea centers, or is it merely due to a partial shocking of bulbar centers?" As has been stated before, it is generally conceded that the polypnea center is cephalically located. However, from the fact that polypnea can be eliminated without cutting tracts and that variable results are produced by lateral lesions, it would seem equally possible for the center to be located in the bulb as well as cephalically.

That shock does enter into the picture is shown by the effect of the medial lesions in cats 49, 53, 55 and 60 and especially the effect of the left lateral lesion in cat 60. Polypnea was completely eliminated by this lesion as shown by the fact that the animal failed to respond to the polypnea tests. Yet the polypnea mechanism partially recovered since four hours later the heat beam and stimulation of the sciatic stump elicited characteristically the short respiratory excursions described above (refer to D, Plate III).

That the short excursions are the result of a partial destruction of the polypnea mechanism is strongly suggested in cat 28 since the mechanism was not any further recovered at 8:50 p.m. than it was at 12:30 p.m. If the short excursions were the result of partial shock, and since recovery from shock occurs, it would be justifiable to expect the mechanism to have further recovered in eight hours. It is of interest to note here also that the central polypnea mechanism was partially eliminated since the animal (cat 28) succumbed at 43.0°C. rectal temperature.

Although the partial elimination of polypnea is associated with a unilateral lesion, a typical polypnea response still can be obtained through the sciatic stump with the stem hemi-sectioned. This is shown in tracings of cats 50 and 51. The fact that this response has not been obtained after the opposite hemi-section lends support to the argument that the reflex polypnea center is cephalically located.

C. The ordinary quiet respiratory mechanism. That the midbrain plays some part in the tonic control of ordinary quiet respiration is generally conceded because of the experimental fact that in completely sectioning the stems at any level caudal to the superior colliculi and cephalad to the acustic stria a characteristic slowing of respiration invariably occurs. This fact was observed as early as 1880 by Markwald who also stated that if, following such a section, the vagi were cut, the respiration became convulsive ("inspiratory cramp") and the rabbit soon died. Coombs and Pike (1918) demonstrated that sectioning the dorsal cervical roots produced the same effect as cutting the stem, and that after sectioning the dorsal roots, sectioning the stem had no effect on respiration. They therefore conclude that afferent impulses pass cephalad through the medulla. Trevan (1916) advanced the hypothesis that the cephalic center is concerned with direct chemical stimulation whereas the bulbar center is concerned with vagal impulses. The work of Trevan (1920) and Lumsden (1923) would place the cephalic centers in the cephalic pons. Lumsden has stated that it is impossible for a cat to continue coördinated respiratory movements if the cephalic pons is not intact.

Coombs and Pike's experiment would seem to eliminate the possibility that the slowing of respiration resulting from a cephalic lesion is due to shock since, if shock were concerned, a slowing of respiration would be produced after the dorsal thoracic roots had been cut off as well as before they had been cut.

In the light of the above work it is interesting to note the following experimental observations with reference to the experiments reported in this paper.

First, that after polypnea was eliminated in these animals (cats 50, 27, 23) by a unilateral lesion, the opposite unilateral lesion slowed the ordinary quiet respiratory movements further in exactly the same manner as in a complete section of the stem.

Second, that in cat 23 coördinated respiratory movements were maintained after lateral stems were sectioned and vagi were cut until the animal was killed by over-heating. It is to be noted that the right lateral cut was at the cephalic level of the pons. On the other hand, in cat 27 with the lateral stems cut at the middle level of the pons, coördinated respiratory movements were eliminated when the second vagus was cut.

Third, that in cat 49 with both vagi and the right lateral stem cut at the middle level of the pons, regular and coördinated respiratory movements were present. Cutting the left lateral stem eliminated respiratory movements. This lends evidence in favor of the opinion that with the vagi cut, the cephalic pons is necessary in order to maintain coördinated respiration, and furthermore, that this mechanism as well as the polypnea mechanism is confined to the lateral stems, and in addition, shows that respiration can be maintained with both vagi and one lateral stem cut. That tonic respiratory impulses reach the cephalic pons from below is further suggested by accelerated respiratory response obtained in cats 50 and 51 when the sciatics were stimulated after a hemi-section of the brain-stem.

SUMMARY AND CONCLUSIONS

1. An operative method is described by which the floor of the fourth ventricle can be exposed without interfering with the respiratory or vasomotor mechanisms.

2. Special attention is called to the fact that reflex polypnea is dealt with under the experimental conditions used.

3. It is pointed out that the *light anesthesia polypnea mechanism* is very sensitive to surgical manipulation; that it can be entirely eliminated without affecting the *ordinary quiet respiratory mechanism;* and that the region about or just caudal to the inferior colliculi is especially sensitive.

4. Polypnea is not dependent on centers situated in the reticular formation, or on fiber tracts crossing the mid-line or passing cephalad or caudad through the medial stems of the isthmus, pons, or upper medulla.

5. If polypnea is dependent on cephalic centers, the centers as well as the fiber tracts passing to and from the centers are located in the extreme lateral stems of the pons and upper medulla. In this connection it is pointed out that although lesions that cut the lateral stems eliminate polypnea this does not necessarily mean that anatomical structures concerned with respiration are destroyed.

6. It is demonstrated that the typical respiratory acceleration response that can be obtained through a sciatic during light anesthesia can still be obtained with the brain-stem hemi-sectioned through the upper medulla level. Section of the opposite side of the stem eliminated the response.

7. The occurrence of short rapid respiratory excursions that are super-

imposed upon the inspiratory phase of the slower ordinary respiratory excursions as a result of unilateral lesions are described and discussed. It is demonstrated that these short excursions are elicited reflexly through a sciatic stump or by means of a heat beam.

8. The following observations are reported in regard to ordinary quiet respiration:

a. Sectioning only one lateral stem through the pons region produces a slowing of the respiratory rate in the same manner as sectioning the whole brain-stem at this level. Sectioning the opposite lateral stem after the first has been sectioned causes a still further slowing of the respiratory rate.

b. An animal can respire in a perfectly normal and coördinated manner with vagi cut and with one lateral stem sectioned through the caudal region of the pons. In the cases observed, section of the opposite lateral stem at this level eliminated coördinated respiratory movements.

I wish to express my indebtedness to Mr. B. R. Macmillan for the making of instruments, photographing and for many helpful suggestions rendered during the progress of this work. I further wish to express my appreciation to Dr. J. W. Papez and Dr. J. A. Dye for their unlimited encouragement. The description of the reticulo-spinal tracts in the cat by Doctor Papez was the impetus for the work reported here.

The expenses of this investigation were defrayed by grants from the Sarah Manning Sage Research Fund.

BIBLIOGRAPHY

Allen, W. F. 1927. Journ. Comp. Neurol., lxiii, 451.
Bazett and Penfield. 1922. Brain, xlv, 185.
Coombs and Pike. 1918. This Journal, xlv, 569.
Garrelon and Langlois. 1907. Journ. de Physiol. et de Pathol. Gen., ix, 640.
Henderson, Y. 1910. This Journal, xxv, 385.
Lumsden. 1923. Journ. Physiol., lviii, 81.
Markwald and Kronecker. 1880. Arch. f. Anat. und Physiol., 443.
Nikolaides, R. and S. Dontas. 1911. Zentralbl. f. Physiol., xv, 192.
Papez, J. W. 1926. Journ. Comp. Neurol., xli, 365.
Richet, C. 1898. Chaleur. Dict. de Physiol., iii, 178.
Sherrington, C. S. 1924. Journ. Physiol., lxviii, 405.
Trevan, J. W. 1916. Journ. Physiol., l, 43.
Trevan and Boock. 1922. Journ. Physiol, lvi, 331.

THE EFFECT OF SILVER NITRATE, GOLD CHLORIDE AND ADRENALIN ON THE SIZE OF THE ENDOTHELIAL CELLS OF ARTERIOLE, CAPILLARY AND VENULE

PAUL EATON AND JOSEPH KRAFKA, JR.

From the University of Georgia, Medical School, Augusta

Received for publication April 24, 1929

Endothelial activity, as a factor in the maintenance of blood pressure, both general and local, is of extreme importance. This is shown by the effect of histamine, a drug capable of bringing about a marked fall in blood pressure, shock, and death, although motor in its action on the arteriole musculature (Dale and Richards, 1919). The seat of action is considered to be the wall of the capillary whose extreme dilatation produces a peripheral congestion or stasis, the effect of which cannot be compensated for by arterial constriction. The similarity between histamine shock and anaphylactic and traumatic shock indicates the practical significance of the problem.

Rouget in 1873, described certain pericytes or primitive muscle cells, which he considered functional in reducing the capillary bore. Clark and Clark (1925) by direct observation, however, have shown a type of activity of the endothelial cells, independent alike of passive pressures and pericytes and subject to temperature influences. Bensley and Vintrup (1928) have observed a wave of contraction initiated by the pericyte passing along the capillary wall. Steinach and Kahn (1903) have controlled local capillary contraction by stimulation of the nerves distributed to the region. Thus while an activity on the part of the endothelium of the capillary is certainly established, probably no direct observations on the endothelium of arteriole and venule have been made.

The differential action of various drugs on the three regions of the vascular bed has been studied by several investigators. Hunt (1927) has shown a dilatation of capillaries after acetyl cholin, an effect which could be prevented by atropin, hence suggestive of autonomic control. Dale (1919) considers the effect on the capillary to be passive as a result of arteriole activity. In the case of adrenalin and histamine, however, he has shown that capillary dilatation takes place in the leg, even after the nerves distributed to this part have been cut, hence the drug must act directly on the endothelium. Rich (1921), in studies on the effect of histamine on capillary activity, discovered the interesting fact that capillary dilatation

and splanchnic congestion would follow the mere handling of the intestines. This dilatation was so complete that the local injection of histamine had no further effect. He also confirmed the observation of Krogh (1920) that a considerable portion of the capillary bed is normally occluded, and that only by proper stimulation do the vessels become patent. Lastly,

TABLE 1

Showing the distribution of area of endothelial cells in arteriole, capillary, and venule as affected by toxic doses of silver-nitrate, gold chloride and adrenalin

AREA	AFTER $AgNO_3$ 2 CC. 1 PER CENT			AFTER $AuCl_3$ 2 CC. 1 PER CENT			AFTER ADRENALIN 15 CC. 1/10,000		
	Arteriole	Capillary	Venule	Arteriole	Capillary	Venule	Arteriole	Capillary	Venule
sq. mm. $\times$ *1500*^{2}									
100–199	5	5		2					
200–299	25	31	7	7				2	
300–399	53	38	25	21	6		4	17	
400–499	16	21	45	21	22	1	26	37	
500–599	1	4	16	21	29	6	32	26	
600–699		1	5	17	17	14	25	16	
700–799			2	7	12	30	7	1	
800–899				3	10	14	6	1	
900–999				1	3	12			
1000–1099					1	12			1
1100–1199						2			
1200–1299						4			1
1300–1399						2			2
1400–1499						1			7
1500–1599						1			15
1600–1699									10
1700–1799									15
1800–1899									17
1900–1999									9
2000–2099									9
2100–2199									4
2200–2299									2
2300–2399									2
2400–2499									4
2500–2599									1
2600–2699									1

the results obtained by Huebner (1907) led him to classify gold and arsenic as capillary poisons.

The present study is of interest in three connections: 1, as demonstrating a normal variability in cell size under constant conditions of the vascular channel; 2, a difference of cell size in arteriole, capillary and venule; 3, a differential effect on cell size of various drugs, indicative of a further independence of activity of endothelium in the three parts of the vascular bed.

METHOD. Cats, varying in weight from 1.8 to 2.2 kgm., were prepared under urethane anesthesia, for blood pressure and respiration tracings. Toxic doses of the drugs to be tested were injected by way of the femoral

TABLE 2

Comparison of lengths, greatest widths, and mean area of endothelial cells in arteriole, capillary and venule as affected by toxic doses of silver nitrate, gold chloride and adrenalin

		AFTER $AgNO_3$	AFTER $AuCl_3$	AFTER ADRENALIN
Arteriole	Length, mm. × 1500	85.1	112.5	92.95
	Width, mm. × 1500	6.65	8.6	8.2
	Area, sq. mm. × 1500^2	333.0	489.0	567.0
Capillary	Length	67.4	91.0	71.3
	Width	8.25	9.97	12.2
	Area	341.0	601.0	490.0
Venule	Length	62.5	69.75	100.15
	Width	11.3	19.35	37.2
	Area	443.0	855.0	1806.0

TABLE 3

Areas of endothelial cells $(\times 1500)^2$

		SILVER NITRATE	GOLD CHLORIDE	ADRENALIN
Arterioles	M	333 ± 5 sq. mm.	502 ± 11 sq. mm.	573 ± 8 sq. mm.
	S D	79 sq. mm.	162 sq. mm.	119 sq. mm.
	C V	24 per cent	32 per cent	21 per cent
Capillaries	M	341 ± 6 sq. mm.	604 ± 11 sq. mm.	494 ± 7 sq. mm.
	S D	98 sq. mm.	156 sq. mm.	109 sq. mm.
	C V	29 per cent	26 per cent	23 per cent
Venules	M	443 ± 7 sq. mm.	852 ± 14 sq. mm.	1814 ± 20 sq. mm.
	S D	103 sq. mm.	208 sq. mm.	293 sq. mm.
	C V	23 per cent	24 per cent	16 per cent

M = Mean. S D = Standard deviation. C V = Coefficient of variation = S D/M.

These frequency distributions are so nearly normal that they merit little discussion. As shown in table 1, the respective group of measurements vary among themselves only as might be expected. The statistical constants presented in table 3 are printed largely because they were worked out.

cannula, and after complete circulatory failure, $AgNO_3$, 0.75 per cent was irrigated through under a pressure of about 50 cm. of water. The cat was then placed in the ice box from two to four hours at the end of which time

several loops of intestine were tied off, removed and immersed in $AgNO_3$ for twenty-four hours, in the dark. Small bits of spread mesentery were then exposed to bright sunlight until black. These were dehydrated in 95 per cent alcohol and mounted in "euparol." Camera lucida drawings were made of the individual cells with a magnification of 1500. These drawings were measured with a planimeter, graduated to read in square millimeters. One hundred measurements were made in each class. The data are treated statistically and presented in tables 1 to 3.

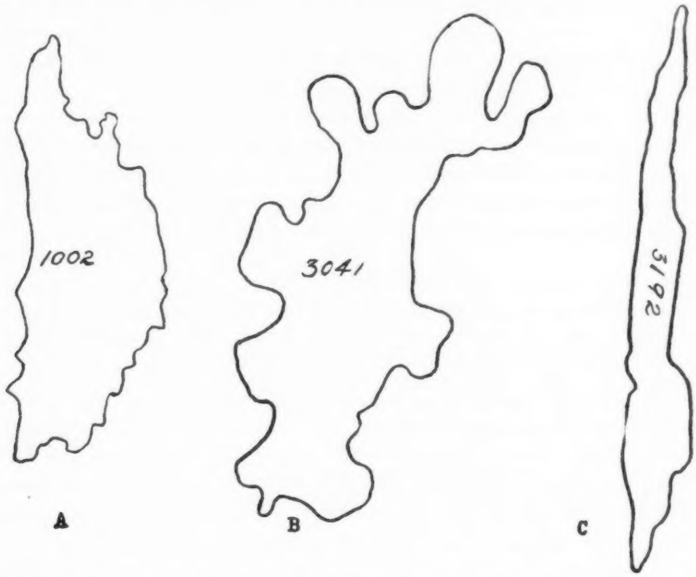


Fig. 1. Camera lucida drawings of endothelial cells. 1500 ×. *A*, venule cell after gold chloride; *B*, venule cell after adrenalin; *C*, arteriole cell after adrenalin.

A comparison of the average area, length and maximum width of endothelial cell in arteriole, capillary and venule when subjected to silver nitrate only, is interesting in that it shows an increase in area associated with an increase in width. The increase in area under gold chloride again is associated with an increase in width of the cells, consistent with an increased dilatation. It will be noted, however, that there is an increase in the arteriole and venule as well as in the capillary, hence the gold seems to be a general endothelial poison and not entirely a capillary poison. Adrenalin produces an interesting effect on the endothelium, while the musculature shows circular constriction as easily seen by the negative picture of these cells in the preparations. No logical explanation of the contraction of the

capillary cells under adrenalin is at hand. The cells widen, but at the same time shorten to reduce the surface area, an effect which is readily possible in the thin walled capillary. The fact in itself is indicative of an independent activity of the endothelium, since a passive dilatation due to vascular pressure, should produce a larger area than shown by the gold if the increase in size of the venule endothelium is produced by the same factor.

The amount of change in size of the endothelium is of interest when compared to the change in capillary bore that Steinach and Kahn were able to produce by nervous stimulation. Observed changes in their material were more than double, or specifically from 18.5 to 7.8 micra.; while our observations involve a threefold change in surface width.

The peculiar picture produced by the adrenalin (arsenic gives a similar effect) particularly on endothelium, is suggestive of the nature of the change. The characteristic form, as shown in figure 1, presents a series of large lobate interlocking processes, such as might be produced by local decrease in surface tension, or a change from a gel to sol state.

BIBLIOGRAPHY

Bensley, R. R. and B. J. Vintrup. 1928. Anat. Rec., xxxix, 37.
Clark, E. R. and E. L. Clark. 1925. Amer. Journ. Anat., xxxv, 265.
Dale, H. H. and A. N. Richards. 1919. Journ. Physiol., lii, 355.
Hooker, D. R. 1921. Physiol. Rev., i, 112.
Huebner, R. 1907. Arch. Exper. Path. u. Pharm., lvi, 370.
Hunt, R. 1917. This Journal, xlv, 231.
Krogh, A. 1920. Journ. Physiol., liii, 399.
Rich, A. R. 1921. Journ. Exper. Med., xxxiii, 287.
Steinach, E. and R. H. Kahn. 1903. Pflüger's Arch., xcvii, 105.

REGULATION OF RESPIRATION

XXXII. The Site of Action of Sodium Sulphide as Related to Peripheral Chemical Control of Pulmonary Ventilation

ARTHUR NACK and GEORGE WULP

From the Physiology Department, University of Michigan, Ann Arbor

Received for publication April 8, 1929

The purpose of the present experiments is to gain further evidence on the probability of peripheral chemical control of pulmonary ventilation as an adjunct to the well accepted central chemical control. Toward this end we have attempted to analyze the stimulating action of sodium sulphide when injected into the circulating blood.

According to Haggard (1921), injection of sodium sulphide gives rise to the formation of hydrogen sulphide in the blood by the process of hydrolysis. The hydrogen sulphide thus liberated is responsible for the respiratory stimulation. Since hydrogen sulphide does not combine with hemoglobin and since it is rapidly oxidized into non-toxic compounds (Haggard, 1921) the stimulating effects are transitory and may be repeatedly elicited with relatively non-injurious effects.

Haggard and Henderson (1922) concluded that the stimulating action of sodium sulphide was not related to changes in the hydrogen ion concentration of the blood and stated as a result of intravenous injection of sodium sulphide in the intact and vagotomized animal that the apparent point of stimulation is at the vagal endings in the lungs. The possibility occurred to us that the absence of stimulation which Haggard and Henderson noted after vagotomy might have been due to a rapid detoxication of the sodium sulphide. The central effect might thus not manifest itself. In view of this and of the work of Gesell (1928) on intra-arterial injection of sodium cyanide it was thought that similar manipulation with sodium sulphide would be of interest.

Method. In general the methods used in our experiment were as follows; both male and female dogs of various breeds were used varying in weight from 7.5 to 15 kilograms. The animals were under morphine and urethane anesthesia during the experiment. The trachea was cannulated and attached to a rebreathing tank containing room air. The carbon dioxide of the expired air was removed by a soda lime cartridge and the oxygen consumed was replaced from time to time to maintain the composition of

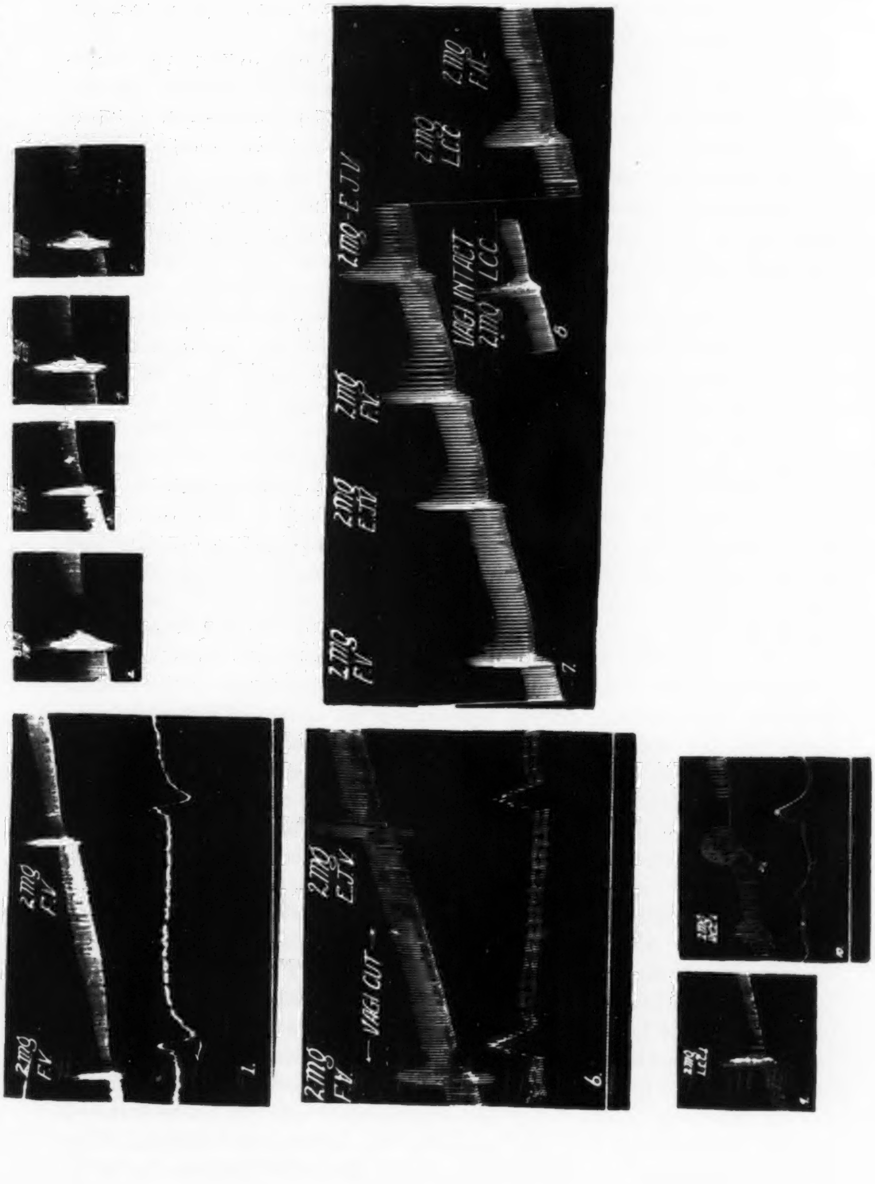
the respired air. A record of the respiratory movements was made from a spirometer on the tank. The vagus nerves, the carotid arteries, and external jugular veins were exposed. The length of exposure of the carotids varied depending upon whether injections were to be made into the common carotid, the internal carotid, or the vertebral arteries. The femoral artery was exposed on one side for the registration of blood pressure, and on the other side both the artery and the vein were exposed for injections. When the abdominal aorta was exposed, it was done by means of an incision along the edge of the spinal muscles, behind the left kidney. The aorta was reached retroperitoneally with little disturbance of the viscera.

The standard injection was 2 mgm. of sodium sulphide in 1 cc. of isotonic salt solution per kilo of dog. Injections were made usually from a 1 cc. Luer syringe with a very fine needle (N. 26). Oblique insertion of the needle in the arteries prevented hemorrhage on withdrawal. A total of 395 arterial and venous injections was made on 19 different animals.

Results. Standard intravenous injection of sodium sulphide by way of the femoral or external jugular veins invariably stimulated respiratory movements. The results of such injection with the vagus nerves intact are shown in figures 1 and 3. Such injection may or may not be followed by apnea. As a rule the larger the injection the greater the stimulation and the greater the tendency towards subsequent apnea (see figs. 2, 3, 4 and 5).

After obtaining such effects with the vagus nerves intact they were blocked or sectioned and the injection repeated. In every instance we were able to demonstrate hyperpnea which was commonly followed by a period of decreased ventilation. In general the hyperpnea was not as violent but was of longer duration than that observed after similar injection with the vagus nerves intact. Record 6 shows typical results obtained in the vagotomized animal following intravenous injection. According to Haggard and Henderson, after section of the vagi injections of sulphide in moderate amounts do not augment respiration and the action of high concentration upon the center is generally depressant. In only one of many experiments was an augmentation of respiration obtained by injection of a large dose of sodium sulphide after section of the vagi. Our experiments on the other hand suggest the possibility of central stimulation of sodium sulphide.

Attacking the problem from another angle, we made injections into the carotid artery first with the vagi intact and next with them severed. In every instance a typical hydrogen sulphide stimulation resulted. The shortness of the latent period (2–4) seconds compared with the protracted period following intravenous injection (12–15) is suggestive of immediate action of sulphide at the centers. It is worthy of note that the magnitude of the effect may be less with arterial than with intravenous injection.


2mg F.V
2mg F.V
VAGI CUT
2mg E.J.V
2mg F.V
2mg E.J.V
2mg F.V
2mg-E.J.V
VAGI INTACT
2mg LCC
2mg LCC
2mg F.A.

Figs. 1–10

Possibly there are two explanations. The time consumed by the transit of sodium sulphide to the brain may be insufficient to permit complete hydrolysis or a large portion of the sulphide may fail to reach the lower center of the brain. Thus Gesell (1928) has found that cyanide injected into the common carotid artery may have little or no effect upon respiratory movements but when the flow is shunted to the lower centers by ligation of the external carotids stimulation becomes pronounced.

Employing the same procedure with sodium sulphide and thus perforce shunting the material into the internal carotid respiratory stimulation was augmented and abrupt in both the intact and vagotomized animal and often when only one-half the standard injection was used (see fig. 7).

Another method of obtaining concentrated action on the center was by injection into the vertebral arteries by way of the central end of the corresponding carotid artery. The carotid artery was occluded approximately one or two centimeters above the vertebral and injected rather forcibly. In this way typical results were produced almost immediately and excessive response was elicited even with half strength solutions. Full strength injections made in this way often produced such a marked paralysis after the hyperpnea that artificial respiration was needed to keep the animal alive. Gesell using sodium cyanide and following this procedure obtained similar pronounced effects (Gesell, 1928). On subsequent releasing of the clamp on the common carotid artery a second typical effect was noted, due to the release of the hydrogen sulphide trapped in the dead end of the artery (see figs. 9 and 10). Injections into the femoral artery produced no or very slight effects depending on the size of the injection. Such results are to be expected from arterial injections in an extremity when one considers that the hydrogen sulphide is rapidly oxidized and detoxicated. It is of interest that similar injections of sodium cyanide produces similar negative effects due to the fixation of cyanide by the tissues (Gesell, 1928).

Since we invariably secured a typical hydrogen sulphide effect with the vagi out it is obvious that, granting a peripheral action, there must also be another point of action. Our results, however, indicate some doubt as to whether there really is an appreciable peripheral action, i.e., on the vagal endings in the lungs. If this peripheral action existed in addition to a central action it should be demonstrable by a study of latent periods. The latent periods following intravenous injections in the vagotomized animal might assumedly be longer, since now the hydrogen sulphide would pass through the lungs and then to the center by the greater circulation before an effect could be produced. But when the latent periods following intravenous injections in the intact and vagotomized animals are contrasted we find that, instead of being longer in the vagotomized animals, they are if anything shorter. The following table shows the difference in latent periods of several injections into two different animals before and after vagotomy.

		Vagi intact Latent period *seconds*	Vagi cut Latent period *seconds*
1	External jugular	13	10
		16	11
		10	
	Average	13	10.5
	Femoral vein	9	13
		18	12
		18	16
	Average	15	13.66
2	External jugular	11	6
		12	7
		9	6
	Average	10.66	6.33
	Femoral vein	15	11
		15	12
		14	9
		11	8
	Average	13.75	10

Such procedure obviously is not without objection for it is conceivable that the sulphide may not reach the vagal endings via the pulmonary circulation but if so we should be able to apply a concentrated flow of sulphide via the nutrient arteries and thus test for peripheral chemical action in another way. Inasmuch as standard arterial injection confined to other regions than the head is without effect (femoral artery) due to the fixation or detoxication of sodium sulphide it is assumed that the absence of effects on direct administration through the nutrient arteries is suggestive of the relative low value of peripheral chemical effect. In order to deliver strong administration via the bronchial arteries without opening the thorax, injections were made with a catheter inserted into the thoracic aorta through either the superior mesenteric or the femoral arteries. These injections were made with the tip of the catheter at different levels in the aorta. No injections were made until the catheter was above the diaphragm and then two injections were made at each centimeter level until the tip of the catheter was well up in the aortic arch. The first injection at each level was 1 cc. and after an interval of 4 or 5 minutes a second injection of 2 cc. was made. We determined the position of the tip of the catheter with relation to the point where the bronchial arteries leave the aorta, after the experiment was completed, by post mortem examination. In this way we could determine very closely where the injections entered bloodstream. Injections which left the catheter more than 2 centimeters below the head arteries generally produced no effect, and when a slight effect was noted it came only after a considerable latent period (45–20 sec-

onds) and after a 2 cc. injection. When, however, the tip was well up in the arch or in the subclavians a typical hydrogen sulphide effect appeared after a very short latent period (2–3 seconds). These effects were the same in the intact and vagotomized animals.

In order to eliminate the possible effect of the sympathetic endings on the lungs, the stellate ganglia on both sides were removed and the vagi severed. After this was done injections were made intravenously into the common carotid and into the catheter. The results were as positive as those obtained in the intact or simply vagotomized animals, that is, a typical hydrogen sulphide effect after a long latent period with intravenous injections, a short latent period after arterial injections, and an effect when injections were made into the catheter only when the tip was high enough for the material to enter the head arteries.

That the vagal endings on the lungs are the point of significant action of hydrogen sulphide is further discounted by the results of our experiments with catheter injections. Were the vagal endings exclusively stimulated by hydrogen sulphide, injections leaving the catheter just above the level of the bronchial arteries would tend to produce an effect after a very short latent period, and after vagotomy no such effect would be noticed. Our results show no effect unless after a long latent period and then only after larger doses than normal.

Furthermore, were the vagal endings on the lungs the point of stimulation the latent periods after intravenous injections should show no variation with the vertebral arteries open or closed. But our results show that with the vertebrals blocked the latent period is slightly longer, as would be expected were the action central rather than peripheral. This difference in latent periods is shown in the following tabulation taken from the records of two different animals:

		Intravenous injections with vertebrals open *seconds*	Intravenous injections with vertebrals closed *seconds*
1	Latent period	10.0	15
		12.0	12
		11.0	
	Average	11.0	13.5
2	Latent period	12	11
		13	15
		10	15
		10	
	Average	11.25	13.66

The generally very much shorter latent period after injections into the common carotid artery than after intravenous injections indicates a point of action in the head, and the rapid and acute effects produced by injections

more directly to the respiratory center (as into the internal carotids or vertebrals) point to the respiratory center as the place of stimulation. This is strengthened by the results obtained by injections via the catheter into the aorta.

SUMMARY AND CONCLUSIONS

The site of stimulation of respiratory movements by sodium sulphide was studied by injection into the femoral and external jugular veins, into the common carotid artery, internal carotid artery, the vertebral artery and the abdominal aorta at, above and below the level of the bronchial arteries.

A total of 395 injections in 19 dogs under varying conditions such as with the vagus nerves intact and cut and with the stellate ganglia intact and removed was made.

Intravenous injection of sodium sulphide elicited temporary stimulation of respiratory movements commonly followed by temporary depression or apnea.

Similar injections into the cerebral arterial circulation produced the same general effects.

The abrupt response following injection into the internal carotid or the vertebral artery, compared with the markedly delayed response to intravenous injection and the absence of response to injection into the external carotid artery indicated central chemical action of sodium sulphide.

The similarity of results obtained by administration of sodium sulphide before and after double vagotomy and double removal of the stellate ganglia suggest the preponderance of central chemical action of sodium sulphide.

The failure to establish a difference in latent periods before and after vagotomy in favor of peripheral chemical stimulation of the afferent endings in the lungs and the failure to stimulate respiratory movements by administration of sodium sulphide into the bronchial arteries is suggestive of the absence or of minor significance of peripheral chemical stimulation.

The results seem to indicate that the probability of central chemical stimulation by sodium sulphide has been established and that peripheral chemical stimulation has not been established.

The indications are of significance in the general problem of central and peripheral chemical control of pulmonary ventilation.

BIBLIOGRAPHY

GESELL: This Journal, 1928, lxxxvi, 164.
HAGGARD: Journ. Biol. Chem., 1921, xlix, 519.
HAGGARD AND HENDERSON: This Journal, 1922, lxi, 289.

STUDIES ON THE CIRCULATION

I. Injection Method: Physical and Mathematical Considerations[1]

J. MURRAY KINSMAN, J. W. MOORE and W. F. HAMILTON

From the department of Medicine and the department of Physiology and Pharmacology: University of Louisville School of Medicine, Louisville, Ky.

Received for publication April 11, 1929

In a previous paper we described a method, based on the injection of a dye into a vein and its recovery from arterial blood, for the simultaneous determination of the pulmonary and systemic circulation times in man or animals and of a figure related to the cardiac output.

Further investigation concerning the cardiac output soon made it apparent that it was desirable to check the principle of the method in this application by the use of artificial models and the accuracy of the method in practice by comparing it in animals with a standard reliable method for determining the cardiac output, such as the direct Fick procedure (Moore et al., 1929). This paper deals with the former problem.

Physical considerations. Our first experiments were concerned with the direct flow of water (distilled) from a reservoir through glass and rubber tubing, into which system glass bulbs, either empty or filled with lead shot or glass beads could be introduced (see fig. 1 inset). The amount of water flowing through the system per minute was measured in a graduated cylinder into which it flowed directly. The dye, phenol-tetraiod-phthalein sodium, Mallinkrodt (Graham et al., 1926), was introduced by a needle puncture through the rubber tubing (I) on the side of the glass tube or bulb nearer the reservoir. The samples were collected in the same way as in the human and animal experiments (using tubes around a Harvard kymograph drum upon which were written sumultaneous records of the time and injection time). The collecting needle, *N*, punctured the rubber tubing on the side of the glass tube or bulb farther from the reservoir. Twenty cubic centimeters of 10 per cent KOH solution were injected at *K* to make the water alkaline. This injection was made slowly and continuously over a period of about one minute. The dye, on the other hand, was injected instantaneously in quantities of either 10, 12.5 or

[1] Expenses of this research were in part defrayed by a grant from the American Medical Association.

25 mgm. dissolved in 1 cc. of water, and the standards for colorimetry were made up from the same solution using certified glassware.

When the water was flowing through straight tubes at a very slow rate the dye assumed a hollow cone-like shape which became more and more

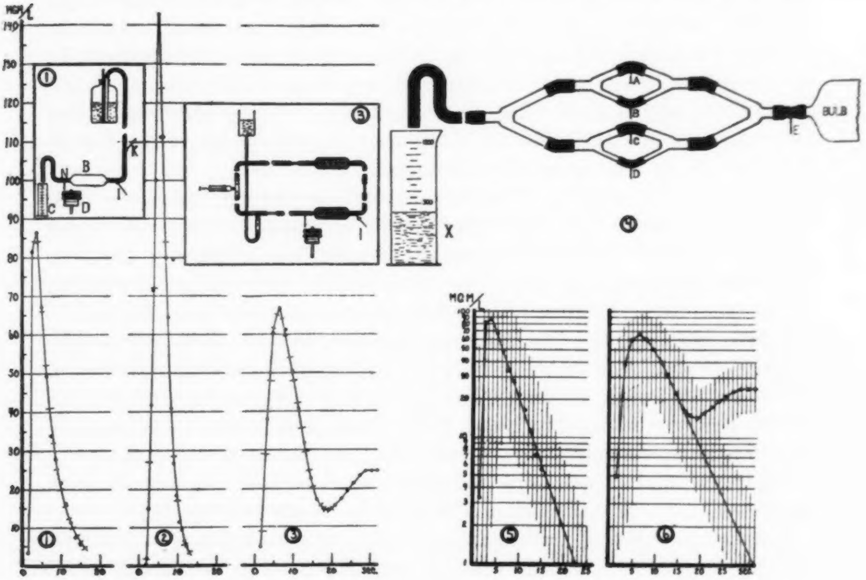


Fig. 1. Time concentration curve of consecutive samples of dye injected at *I* (inset) into water flowing through the bulb *B*. The samples are taken through the needle *N* into the tubes on a rotating kymograph drum *D*.

Fig. 2. Same as figure 1 except that bulb *B* was filled with beads.

Fig. 3. Time concentration curve of consecutive samples taken from an apparatus (inset) designed to simulate the animal circulation.

Fig. 4. Apparatus used in an experiment designed to show that dye concentration in different branches of a stream does not vary significantly. See table 1.

Fig. 5. Same curve as figure 1 plotted on a semi-logarithmic scale. It will be noticed that the downstroke is a straight line.

Fig. 6. Same curve as figure 3, plotted on a semi-logarithmic scale. It will be observed that when the straight downstroke is prolonged, it serves to differentiate once-circulated from twice-circulated dye.

elongated as it advanced, indicating, as would be expected, that the water in the center of the tube flowed very much more rapidly than that at the periphery. This is known in hydraulics as "straight line flow" (Russell, 1925) and would be expected to change into turbulent (eddying) flow in case the water passed a bend in the tube or in case the velocity of flow

exceeded the "higher critical velocity." Our observations indicate that flow at a rate well below the critical velocity becomes turbulent as it passes even a gradual bend in the glass tube. They further indicate that in small straight tubes the size of average arteries, the flow becomes turbulent at velocities far lower than those which would be arrived at by extrapolating from the tables in engineering text books. From considerations of the rate of flow in arteries, the pulsatile changes in pressure, the elasticity of the arterial wall, and the tortuosity and branching of the channel, we are convinced that the flow in the arterial stream is always turbulent. Our experiments were done at a rate of flow that was always above the critical velocity. Flow was thus always turbulent.

After these preliminary observations of flow through tubes we then inserted into the system a glass bulb of 250 cc. capacity filled with water (see fig. 1 inset). The dye was rapidly conveyed to all parts of the bulb and began to leave it at once. It took about 15 to 40 seconds, depending on the rate of flow, for practically all the dye to disappear from the bulb.

When, however, the first bulb was replaced by one filled with beads, to simulate roughly a capillary bed, the manner of flow of the dye colored water was definitely altered. Instead of beginning to leave at once it traveled slowly as a fairly broad band which widened as it progressed. The time of passage of all the dye, however, was definitely decreased.

Figures 1 and 2 represent the concentration-time relations in such experiments. (Fig. 1 bulb without beads; fig. 2 bulb with beads.)

We then constructed the apparatus shown in figure 3 (inset) in which an effort was made to simulate conditions as they exist in the animal body. The fluid was maintained in circulation by a Record syringe whose strokes were counted, thus enabling us to measure the flow per minute. The direction of flow was kept constant by means of valves. Fluid lost in the sampling was replaced from a reservoir guarded by a one-way valve. The elasticity of the arteries was simulated by the rubber tubes shown in the figure. The curves obtained were similar to figure 3 in all respects. A secondary wave will be noticed, and it was observed that this corresponded in time to the recirculation of dye as seen through the glass tubing. This wave is similar to the wave seen in animal and human experiments and undoubtedly due to the same causes.

The question naturally arose as to whether our method of collecting samples of the fluid while continuously flowing, gave an accurate representation of the changing conditions as they actually existed in the body of the fluid itself—in other words, whether the changing concentration of dye in the samples, reflected synchronous and identical changes in the concentration of the dye in the fluid as it passed any given point. The obvious procedure to determine this relationship would be to collect consecutive samples through a needle inserted very close to the outlet of the

tube, at the same time allowing all of the water to flow into larger tubes consecutively, in the same manner as for the samples, and to compare the curves so obtained with each other. For practical purposes, however, we may obtain this information indirectly by collecting one sample from the needle during the exact time the fluid is flowing through the system, and comparing it colorimetrically with the whole amount which flows through. If the needle collects fluid representing an average concentration of the dye in the water in the corresponding cross-section of the tube at any given instant, then the concentration of dye in the sample should be the same as that in the total volume; if, on the other hand, the water collected by the needle comes from a point in the cross-section of the tube in which the dye-concentration is either higher or lower than that for the whole cross-section, the concentration in the sample would be higher or lower than that in the total volume. Our experiments relative to this point show that the comparative concentration in the sample and the total volume check well within the limits of experimental error. In one case the concentration of dye in the sample was 11.5 mgm. per L.; the observed concentration in the total volume was 11.0 mgm. per L.; while the calculated concentration in the total volume (based on the assumption that 100 per cent of the dye has passed through during the period of observation) was 11.5 mgm. per L.

A question of even greater importance was raised; are samples taken from tubes of different diameters comparable, no matter how large or how small the tube from which the collection is made? This has its counterpart in the arteries, the ones which are available for puncturing (e.g., the femoral, brachial and radial) being of different sizes; hence it appeared of considerable importance to know whether we could expect correspondingly reliable results whether we puncture a large or a small tube or artery. To determine this point, we arranged our apparatus as illustrated in figure 4 in which the Y tubes were of different calibers. Using needles identical in size for A, B, C and D, but larger for E, the volume of water obtained simultaneously over a given period of time was as shown in table 1.

This table also illustrates the results from a series of such experiments, the two intermediate needles being used in only one experiment. The concentration of dye in the total volume and in the sample from each needle was observed colorimetrically, and also calculated, as explained above, on the assumption that 100 per cent of the dye goes through during the experiment. It is apparent that the error is well within the experimental limit (of colorimetry, etc.). We can reasonably assume, then, that it makes no difference from what size of tube the sampling is done. By analogy, therefore, we believe that in man there is no difference as far as accuracy is concerned, whether we puncture the relatively large femoral or the relatively small radial artery. Moreover, there is no difference whether we use a large or a small needle.

TABLE 1

Comparison of the concentration of dye in the output of needles of various sizes (A, B, C, D, E, fig. 4) with the observed and calculated concentration in the total flow, X

SOURCE	EXPERIMENT P			EXPERIMENT Q			EXPERIMENT R			EXPERIMENT S			EXPERIMENT T			EXPERIMENT U			EXPERIMENT V			EXPERIMENT W		
	Flow	Concentration		Flow	Concentration		Flow	Concentration		Flow	Concentration		Flow	Concentration		Flow	Concentration		Flow	Concentration		Flow	Concentration	
		Observed	Calculated		Observed	Calculated		Observed	Calculated		Observed	Calculated		Observed	Calculated		Observed	Calculated		Observed	Calculated		Observed	Calculated
	cc.	*mgm. per liter*	*mgm. per liter*	*cc.*	*mgm. per liter*	*mgm. per liter*	*cc.*	*mgm. per liter*	*mgm. per liter*	*cc.*	*mgm. per liter*	*mgm. per liter*	*cc.*	*mgm. per liter*	*mgm. per liter*	*cc.*	*mgm. per liter*	*mgm. per liter*	*cc.*	*mgm. per liter*	*mgm. per liter*	*cc*	*mgm. per liter*	*mgm. per liter*
X	1013	8.0	9.5	1008	9.0	9.6	1093	12.2	12.2	1200	10.4	11.1	1150	9.6	10.5	914	16.3	16.9	976	9.0	8.9	1075	8.2	8.1
A	12	6.9	9.5	12	9.0	9.6	4	12.5	12.2		11.4	11.1		10.0	10.5	9	16.5	16.9	10	8.8	8.9	9	8.3	8.1
B	7	7.7	9.5																					
C	14	8.2	9.5																					
D	4	8.8	9.5	4	8.3	9.6	4	13.5	12.2		11.1	11.1		10.8	10.5	9	16.4	16.9	7	8.5	8.9	10	8.1	8.1
E							6	11.9	12.2							118	16.7	16.9	127	10.0	8.9	136	8.3	8.1
	Steady stream																		Pulsatile stream					

Mathematical considerations. Since in calculating the flow through the system we are dependent upon observations which tell us the extent to which the dye has been diluted by the water, we must in some way account for all or practically all of the dye which has been injected. We must also guard against using the same dye twice, which at first sight would seem difficult when it recirculates.

In the straight-flow experiments, in which the water is not recirculated (figs. 1 and 2), the curve as actually determined, approaches the base-line (zero concentration) so closely as to account for practically all the dye injected, the remainder being so small that for practical purposes it may be ignored. Of course, the curve approaches the base-line as a limit and would actually reach it only at infinity—a matter of further importance. In this type of experiment, therefore, all of the dye is accounted for without further ado.

When, however, samples are taken from apparatus in which the water recirculates (as does the blood of animals) there appears a secondary wave (due to recirculation) which hides the end of the primary curve. This secondary wave introduces difficulties; for if we include this curve too, in accounting for the dye, we are obviously using some of the dye twice; if, on the other hand, we disregard it entirely, we are eliminating with it some of the dye on its first circulation. We must, therefore, in some way differentiate once-circulated from twice-circulated dye.

The extent to which this factor interferes may be illustrated by the experiment represented by figure 3, which shows such a curve closely resembling those obtained in animal experiments. In this experiment, after the colorimetric readings had been made, equal quantities from each sample from the beginning of the primary wave to the beginning of the secondary wave, were mixed together and the concentration determined. This "observed" concentration was 34.5 mgm. per liter. In the 19.2 seconds represented by these samples, the measured flow was 247 cc. At the above concentration, this would account for 8.52 mgm. of the dye. Since 10 mgm. were injected, there remains 1.48 mgm. buried under the secondary wave. This introduces, in this particular experiment, an error of 14.8 per cent, which would be greater or smaller in other experiments, depending upon where the secondary wave happened to occur.

It would be of obvious importance, therefore, to find some means of mathematically prolonging the primary curve so that all of the dye on its first circulation might be accounted for. The curve could be most conveniently prolonged if a method of plotting were found which made the descending limb a straight line. Such a method has been developed, based on the fact, noted above, that the time of recovery of all of the dye approaches infinity. This at once suggests a logarithmic scale. Figure 1 represents a curve from a straight flow experiment, plotted on a linear

scale; the down stroke is concave to the right. Figure 5 represents the same curve plotted on a semi-logarithmic scale, whose abscissae (time) are linear and whose ordinates (concentration) are logarithmic; the downstroke is a straight line. The curve of figure 3, obtained from a re-circulation experiment and plotted on a linear scale, is replotted in figure 6, on a semi-logarithmic scale; the downstroke is likewise a straight line. Moreover, it will be noted that the beginning of recirculation is sharply indicated by the departure of the observed curve from this straight line. When plotted on a linear scale, however, the beginning of recirculation is hidden by the natural concavity of the down-stroke.

This method of plotting has been repeated in all of our experiments (straight flow, recirculation and animal) and always produces a straight line. Moreover, we believe that its principle is mathematically sound.

Since the down-stroke, thus plotted, is a straight line, as soon as its direction is determined by three or more points, it is prolonged as far as desired, and the secondary wave, when present, ignored, since that portion of the dye which is making its first circulation and is buried under it can now be accounted for. Because the curve is infinite in extent, we cannot include all the points upon it, but for practical purposes we include only a sufficient number to bring our results within the limits of experimental error. In these water experiments where a relatively small amount of the dye is injected, to obtain sufficient accuracy, we have found that the curve need be prolonged no farther than the 1 mgm. per L. line. In the animal experiments, however, where a very large amount of dye is injected, we have found it unnecessary to prolong it farther than the 10 mgm. per L. line.

Having thus accounted, either by observation or by mathematical procedures, for practically all of the dye injected, there remains one other factor necessary for calculating the flow. This is the determination of the average concentration of the dye in the water flowing during the period of time occupied by the primary curve. This can best be illustrated by reference to figure 1. In this experiment, the dye came through in appreciable quantities for 15 seconds. The consecutive samples were analyzed (and the curves drawn (figs. 1 and 5) according to the methods outlined above), and then equal quantities from each were thrown together and the concentration of this mixed sample determined. This was found to be 33.3 mgm. per L. During these 15 seconds, 0.710 liter flowed through the system. If all the 25 mgm. of dye were mixed uniformly with this quantity of water, the dye would be diluted to a concentration of 25 mgm. per 0.710 L., or 35.2 mgm. per L.—a difference of 5.4 per cent from the observed concentration. If we take the average of the concentration values on the linear curve, at second intervals, we get the figure of 32.5 mgm. per L., in close agreement with the observed concentration, though 7.7 per cent off from the calculated or theoretical concentration.

Because of its nature, for strict mathematical accuracy, the curve

should be integrated, but the mathematical operations of integrating the whole curve are so complicated that for practical purposes it was thought best to average readings taken from it at finite intervals. A convenient interval is one second. A shorter interval might have been selected, except that its use would involve more labor, and moreover, the error introduced by the larger interval on the downstroke is partly cancelled by an opposite error on the upstroke.

Now, from observation of the same curve plotted on semi-logarithmic paper (fig. 5), it is seen that the prolonged downstroke cuts the 1 mgm. per L. line at 23 seconds—a total duration of 22 seconds between the beginning and the end of the arbitrarily limited curve. During this time 1.041 liters of water flowed through the system. If all the 25 mgm. of dye

TABLE 2

Comparison of the measured with the calculated flow through straight systems and through closed systems in which the water recirculates

EXPERIMENT	FLOW PER MINUTE		ERROR	
	Measured	Calculated		
	liters	*liters*	*per cent*	
1 straight flow	1.154	1.123	−2.7	
2 straight flow	1.150	1.171	+1.8	
3 straight flow	1.156	1.185	+2.5	+2.4
4 straight flow	1.012	1.046	+3.4	
5 straight flow	1.040	1.096	+5.4	
6 straight flow	1.105	1.137	+2.9	
AA recirculation	0.772	0.866	+10.9	
EE recirculation	0.772	0.740	−4.1	+4.8
GG recirculation	0.796	0.848	+6.5	
HH recirculation	0.537	0.568	+5.8	

had been mixed uniformly with this amount of water the average concentration would have been 24.0 mgm. per L.

Averaging the concentration from this curve in the manner outlined above, the figure of 22.1 mgm. per L. is obtained—a difference of 7.9 per cent from the theoretical average concentration.

Thus, in this experiment, the average concentration as estimated by any of the three methods—determined by colorimetry of mixed samples, calculated from the curve on linear scale, or calculated from the prolonged curve on semi-logarithmic scale—is close enough to the theoretical average concentration to be within the limits of experimental error. The first two could be easily applied to the straight flow experiments, but not to the recirculation experiments because of the dye unaccounted for. The third method, however, is applicable to both types of experiment; and since in

animals and man there is always recirculation, it seems, from the above considerations, that there is ample justification for its use.

Having in this way accounted for practically all of the dye, and having determined the average concentration during the time of passage of the dye, the actual calculation of the flow is clearly possible by the use of the formula

$$\frac{60\,I}{C\,T} = F$$

in which I = mgm. of dye injected
C = average concentration in mgm. per L. during primary curve
T = duration of the primary curve in seconds
F = flow in L. per minute

Table 2 gives the results of six consecutive straight flow and of four re-circulation experiments in which the measured flow is compared with the output as calculated in the above manner. The average error for the straight-flow experiments is + 2.4 per cent and for the re-circulation ones, +4.8 per cent. It appears from this table that there may be a slight systematic error. We feel that this divergence cannot be accounted for on the above mathematical considerations and are inclined to attribute it to an undiscovered technical error. Of course an error of this size is of no consequence in calculating the circulation rate.

SUMMARY

Evidence is presented showing that it is possible to calculate accurately the flow of water through a system of tubes and bulbs, simulating the cardio-vascular apparatus of animals. The calculation is based upon the average concentration and time relations of consecutive samples of the stream into which a dye has been injected.

These samples serve equally well whether taken through large or small needles or from large or small branches of the stream.

Curves obtained in experiments in which the water recirculates are similar to those in animal experiments. The confusion introduced by re-circulation is avoided by plotting the curve on semi-logarithmic paper. The descending limb of the primary wave is then a straight line and when prolonged serves to differentiate once-circulated from twice-circulated dye.

BIBLIOGRAPHY

Graham, E. A., W. H. Cole, G. H. Copher and S. Moore. 1926. Jour. Amer. Med. Assoc., lxxxvi, 467.
Hamilton, W. F., J. W. Moore, J. M. Kinsman and R. G. Spurling. 1928. This Journal, lxxxiv, 338.
Moore, J. W., J. M. Kinsman and W. F. Hamilton. This Journal, lxxxix 331.
Russell, G. E. 1925. Text-book on hydraulics. Ed. III; Henry Holt & Co., pp. 161–163.

STUDIES ON THE CIRCULATION

II. Cardiac Output Determinations; Comparison of the Injection Method with the Direct Fick Procedure[1]

JOHN WALKER MOORE, J. M. KINSMAN, W. F. HAMILTON AND R. G. SPURLING

From the Departments of Medicine and Surgery and the Department of Physiology and Pharmacology, University of Louisville, School of Medicine, Louisville, Ky.

Received for publication April 11, 1929

The methods generally used in calculating the cardiac output in man are indirect respiratory procedures and they have all received a certain amount of adverse criticism. For summary of the literature see Henderson (1923), Wiggers (1923), and Marshall and Grollman (1928).

The disadvantages which are inherent in any respiratory procedure are lacking in an injection method, in which a foreign substance is introduced into the venous stream and the cardiac output calculated from its concentration in arterial blood. This principle has been applied to animals by Stewart (1897, 1921a, 1921b); Henriques (1913), Bock and Buchscholtz (1920), and Gross and Mittermaier (1926).

So far as we have been able to find, the cardiac output in man had never been determined by any application of Stewart's principle until the appearance of recent papers from these laboratories (Hamilton, Moore, Kinsman and Spurling, 1928a, 1928b), in which we described radical modifications of the earlier technique, which removed its theoretical and practical deficiencies. The method thus perfected was then applied to man.

The injection method. A known amount of an easily detectable substance (phenol-tetroid-phthalein sodium, Mallinkrodt (Graham et al., 1926)) is injected quickly into a vein. At a convenient point in the arterial system a puncture is made and consecutive blood samples collected. Knowing the quantity of substance that has been injected and the concentration in the various samples together with the time occupied by the first circulation, the cardiac output per minute is determined.

To test the accuracy of the method we chose two courses of investigation. The first was confined to the determination of flow through mechanical apparatus by our procedure, checking against the flow or output actually

[1] Expenses of this research were in part defrayed by a grant from the Ella Sachs Plotz fund.

measured in graduated cylinders, while the second was carried out in dogs, using a known and accepted method, the direct Fick, as a check.

The results of our first course of investigation are reported in detail elsewhere (Kinsman et al., 1929); suffice it to say, that with the use of apparatus varying from straight tubes of different sizes to a more complex scheme in which the vascular system is, in a way represented, we were able to predict the actual flow (output) with an average error of 3.2 per cent. Thus, having satisfied ourselves of the soundness and accuracy of the principle of the method we turned our attention to checking its accuracy in practice against the direct Fick in dogs.

A Harvard kymograph was placed on a horizontal bar which could be set at various heights and rotated on the upright of a heavy iron stand. Around the circumference, at the top of the drum carrying the usual smoked paper, were placed small, thin-walled, paraffined glass tubes, each about 6 mm. inside diameter and 3 cm. long drawn to a blunt point below. They were held in place by a rubber band; they were further kept from dropping by a fairly heavy rubber tube below them and upon which they rested. This tube also served the purpose of keeping blood from running down upon the paper and signal points below. The latter consisted of 1, a time marker, recording seconds; 2, a signal marker recording the beginning and end of the injection, and 3, a pulse recorder, either a key and signal magnet, worked by hand or a tambour with impulse transmitted from a cup placed over an artery. The speed of the drum was regulated so that each tube took one or two seconds, in different experiments, to pass the sampling needle.

Heparin as an anti-coagulant sprinkled in the paraffined tubes was found less likely to produce hemolysis than powdered oxalate. A small spatula having a capacity of about 1 mgm. was used in measuring the powdered heparin. It was found that this quantity would prevent clotting in the amount of blood collected, provided it was well mixed. Mixing was brought about by a brisk shaking of the drum with tubes in place. On account of hemolysis it was necessary to remove standing drops of blood from the sides of the tubes.

In some of the experiments the dog (narcotized) was suspended above the drum in a jacket with a hole over the heart, while in others the animal was laid on his side close to the edge of an operating table. The cardiac punctures were made under novocaine, and 100 mgm. of heparin injected intravenously to slow clotting. A 15 gauge needle 15 cm. long without syringe adapter was used to enter the left ventricle.

As soon as blood was flowing freely the drum was swung under the needle and started. Immediately the dye was injected into the (exposed, anchored) jugular vein. The strong dye solution (250 mgm. in 1 cc.) of phenoltetroid-phthalein sodium, when drawn up into a precision syringe will dry

on the plunger in a short time and cause it to stick. This was prevented either by wrapping the barrel and plunger in gauze soaked with alcohol, or by placing a drop of sterilized mineral oil on the plunger so that the outer fourth of the barrel was lubricated.

As a result of this procedure we had consecutive samples of the blood carrying the dye. It was possible to determine the time of appearance of the dye and to estimate its concentration in each sample.

To make these estimations we measured in a capillary pipette the same quantity (25 to 50 cmm.) of plasma or serum from each tube and added it to 1 cc. of one per cent alkali in water. The concentration of the substance per liter was determined colorimetrically against standards made by diluting the dye in known quantities of the heparinized blood of the animal. Convenient dilutions are 1:2000, 1:4000, and 1:8000. Hemolysis in these manipulations was avoided by making up to mark in paraffined test tubes. One cubic centimeter or even 0.5 cc. of the alkali-plasma mixture can be handled very satisfactorily in the Bausch and Lomb micro colorimeter.

When the samples have been analyzed and the concentrations in mgm. per L. tabulated they can be plotted either on linear or logarithmic coördinates. In our earlier paper (Hamilton et al., 1928a) we used a linear scale and showed by puncturing both right and left hearts that there is usually a secondary increase in concentration which occurs on the downward slope of the primary wave and is due to recirculated blood carrying dye a second time past the point of sampling. The area under this curve is made up partly by dye on its first circulation and partly by dye on its return flow. Since it is necessary, in calculating the cardiac output, to use the average concentration of the dye on its first circulation exclusively, it is obvious that we must find some way of eliminating the recirculated dye from our calculations, particularly since the concentration and time of appearance of the recirculated dye varies in different experiments. In our earlier contribution we suggested that "it may be possible when the results of further experiments are interpreted to calculate the dye represented in this hidden portion of the (first circulation) curve." This prophecy is fulfilled by the simple expedient of plotting the curve on semi-logarithmic paper. This procedure makes the descending limb of the concentration curve a straight line (fig. 1) up to the time when recirculation begins, and by prolonging this straight line we can determine how much of the dye is on its first circulation (I, fig. 1, expt. 9) and how much is on its second circulation (II, fig. 1, expt. 9).

Thus, knowing the height of the first circulation curve throughout its extent, we can calculate the average concentration of the dye by taking the average of the heights of the ordinates at one-second intervals. From

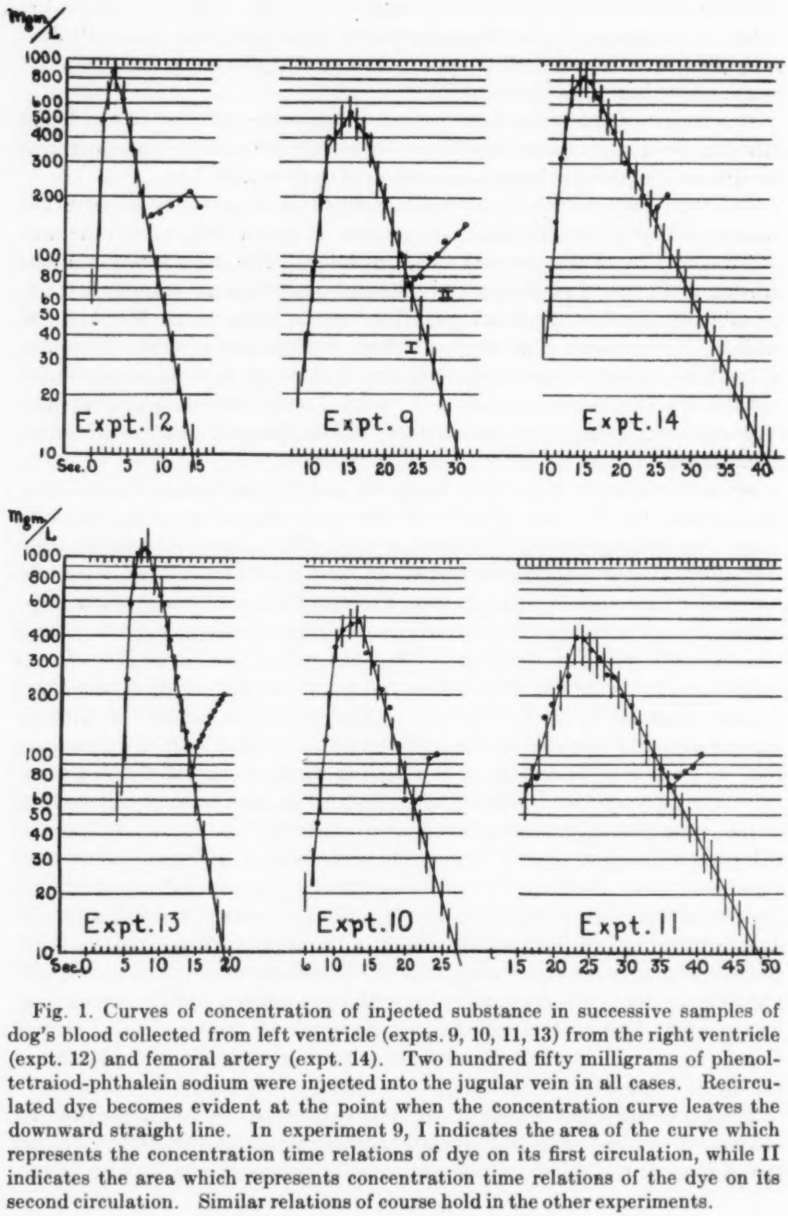


Fig. 1. Curves of concentration of injected substance in successive samples of dog's blood collected from left ventricle (expts. 9, 10, 11, 13) from the right ventricle (expt. 12) and femoral artery (expt. 14). Two hundred fifty milligrams of phenoltetraiod-phthalein sodium were injected into the jugular vein in all cases. Recirculated dye becomes evident at the point when the concentration curve leaves the downward straight line. In experiment 9, I indicates the area of the curve which represents the concentration time relations of dye on its first circulation, while II indicates the area which represents concentration time relations of the dye on its second circulation. Similar relations of course hold in the other experiments.

this average concentration we can calculate the cardiac output, using the following formula:

$$\frac{60\,I}{C\,T} = F \tag{1}$$

in which I = mgm. of dye injected.
C = average concentration in mgm. per L. during the first circulation
T = duration in seconds of primary curve, including its prolongation to (for practical purposes) the 10 mgm. per L. line.
F = flow in L. per minute.

We feel that this procedure is justified in calculating the cardiac output because it is analogous in every respect to the procedure outlined elsewhere (Kinsman et al., 1929), which accurately calculates the flow through artificial systems.

Comparison of figure 6 (Kinsman et al.), obtained from artificial systems with figures presented here, shows the close similarity in detail between the artificial and physiological curves.

Having shown that the injection method is sound on physical and mathematical bases, the next step is to show that in actual practice it gives output figures which check with the results of a known and accepted method, namely, the direct application of the principle of Fick (Zuntz and Hagemann, 1898).

THE FICK METHOD. If we know the oxygen consumption in liters per minute (O) and the amount of oxygen (in liters) taken up by a liter of blood in passing through the lungs ($A - V$), we can calculate the cardiac output in liters per minute (F), by the following formula:

$$\frac{O}{A - V} = F \tag{2}$$

where A = liters of oxygen per liter of arterial blood, and
V = liters of oxygen per liter of venous blood.

In our experiments oxygen consumption was determined by means of a closed circuit metabolic apparatus (Metabolor, McKesson) with a graphic recording device. A plaster of paris mask was used to connect the dog with the apparatus. It was made to fit the dog's snout and sealed with a thick paste of gum tragacanth. Venous blood was obtained by puncture of the right heart; arterial blood by puncture of the left heart or an artery.

The Fick samples were taken in air tight oiled syringes and mixed with potassium oxalate under oil. Their oxygen content was determined with the Van Slyke Neill (1924) manometric apparatus using 2 cc. blood samples. Commercially obtainable saponin proved so weak that it was necessary to use twice the prescribed quantity to obtain duplicate checks (Himwich, 1928).

Comparison of the two methods. The order of procedure in detail was as follows: The animal was completely narcotized with either morphine or sodium barbital. An oxygen consumption curve was continued until it showed a constant slope for at least ten minutes. Next, blood samples were drawn for the Fick. The injection procedure was then carried out and immediately another pair of blood samples was drawn for the Fick. The respiratory tracing was continued throughout these procedures and prolonged until we could be sure that no change in oxygen consumption had occurred. The cardiac output was then calculated by both methods.

Experimental results. A protocol of a single experiment is given in detail below. The results of other experiments are summarized in table 1.

Our first eight experiments had to be discarded because, owing to weak saponin we were unable to get proper checks in our blood gas analysis. Suffice it to say, however, that in each of these eight discarded experiments the output with the injection method was between the minimum and maximum Fick, that is, using the maximum and minimum $A-V$ difference in our calculation.

The six experiments herein reported were done consecutively on three different dogs. It is to be noticed that the average difference (table 1) between the calculated output of the two methods is -4.7 per cent. In one experiment the injection method gave a result 29 per cent lower than the

Protocol of experiment 11. May 8, 1928

Brown hound, weight 24 kgm., morphine 8 mgm. per kgm. Heparin 100 mgm. Heart rate 36. O_2 consumption 0.197 L. per min.

Oxygen volumes per cent			Liters oxygen taken up by 1 liter blood in passing through the lungs
Sample I, left heart	Sample I, right heart	A-V difference	
20.04 19.86	13.72 13.82	6.18	0.0618
Sample II, left heart	Sample II, right heart		
18.19 18.10	11.91 11.75	6.32	0.0632

Output by Fick method (see equation (2)).

$$\frac{0.197}{0.0618} = 3.19 \text{ liters per minute}$$

$$\frac{0.197}{0.0632} = 3.12 \text{ liters per minute}$$

Between taking samples 1 and 2, for Fick, 250 mgm. phenol-tetraiod-phthalein sodium were injected into right jugular vein. The left ventricle was punctured and consecutive samples were taken. Their concentration and time relations are recorded below. The curve (see fig. 1) was plotted and the values in the last two columns below read from it.

TUBE NUMBER	OBSERVED		READ FROM CURVE	
	Time after injection of taking samples	Concentration of dye in each sample	Intersection of second ordinates	Concentration at intersection
	seconds	*mgm. per liter*	*seconds*	*mgm. per liter*
1–14	0–15.4	0	16	61
15	16.5	71	17	80
16	17.6	77	18	103
17	18.7	156	19	133
18	19.8	180	20	175
19	20.9	222	21	230
20	22.0	253	22	300
21	23.1	400	23	390
22	24.2	392	24	400
23	25.3	345	25	360
24	26.4	317	26	325
25	27.5	256	27	293
26	28.6	250	28	263
27	29.7	200	29	230
28	30.8	178	30	198
29	31.9	146	31	168
30	33.0	118	32	142
31	34.1	100	33	122
32	35.2	86	34	103
33	36.3	70	35	88
34	37.4	78	36	75
35	38.5	83	37	64
36	39.6	87	38	55
37	40.7	100	39	46
			40	40
			41	34
			42	29
			43	24
			44	21
			45	17
			46	15
			47	13
			48	11
				33)4608
				139.7

Average concentration of dye in blood passing through the circulation the first time = 139.7 mgm. per liter.

Substituting the values found in equation I, we have

$$\text{Cardiac output } \frac{60 \times 250}{139.7 \times 33} = 3.25 \text{ liters per minute}$$

Injection output = 3.25 liters per minute
Fick output = 3.19 liters per minute
Difference = 0.06 liters per minute
or 1.9 per cent

TABLE 1

Summary of experimental data in which cardiac output figures as determined by the injection method are compared with those as determined by the direct Fick method

EXPERIMENT NUMBER	DOG NUMBER	WEIGHT	O_2 CONSUMPTION	HEART RATE PER MINUTE	BLOOD OXYGEN: Arterial	BLOOD OXYGEN: Venous	BLOOD OXYGEN: Arterio-venous difference	INJECTION FIGURES: Amount of dye injected	INJECTION FIGURES: Average concentration in blood samples	INJECTION FIGURES: Duration of prolonged curve	CIRCULATION: Fick method	CIRCULATION: Injection method	CIRCULATION: Per cent difference	CIRCULATION TIME FROM JUGULAR TO POINT OF SAMPLING	COMPLETE CIRCULATION TIME	DRUGS PER KILO
		kgm.	*cc. per minute*		*vol. per cent*	*vol. per cent*	*vol. per cent*	*mgm.*	*mgm. per liter*	*sec.*	*liters per minute*	*liters per minute*		*sec.*	*sec.*	
9	I	15	158	44	19.99	15.62	4.37	250	189.2	23	3.62	3.45	−4.7	9.3	15.0	Morphine sulphate 10 mgm.
10	II	22	204	45	16.60	11.77	4.83	250	178.8	20	4.22	4.20	−0.5	7.7	14.3	Morphine sulphate 10 mgm.
11	II	22	197		19.95	13.77	6.18	250	139.7	33	3.19	3.25	+1.9	16.5	20.0	Morphine sulphate 10 mgm.
12	III	18	111		19.085	16.28	2.805	250	266.3	14	3.95	4.02	+1.8	1.3*	6.8	Sodium barbital 300 mgm.
13	III	18	121	96	14.13	10.61	3.52	250	435.3	14	3.45	2.46	−29.0	5.3	9.5	Sodium barbital 300 mgm.
14	I	15	121	76	20.30	14.25	6.05	250	243.8	30	2.00	2.05	+2.5	10.8	14.1	Sodium barbital 300 mgm.
Average..........													−4.7			

* Samples taken from right heart.

TABLE 2

Data relating to four human cardiac output determinations

INITIALS	WEIGHT	AGE	SEX	COLOR	PULSE RATE	DIAGNOSIS	DYE INJECTED	DURATION OF PROLONGED CURVE	AVERAGE CONCENTRATION IN BLOOD SAMPLES	CIRCULATION	CIRCULATION TIME FROM ANTECUBITAL VEIN TO FEMORAL ARTERY	TOTAL CIRCULATION TIME
	kgm.						*mgm.*	*seconds*	*mgm. per liter*	*liters per minute*	*seconds*	*seconds*
X.........	70	35	M.	W.	54	Normal	1,000	38	232	6.81	16.4	17.2
N. H......	57	65	F.	W.	72	Pernicious anemia; red blood cells 2,800,000; hemoglobin 60 per cent	1,000	31	149	6.56	10.8	
C. H.......	56	40	M.	W.	71	Pernicious anemia; red blood cells 800,000; hemoglobin 30 per cent	1,000	16	313	12.25	8.5	8.4
I. R.......		35	M.	W.	75	Normal	1,000	37	275	5.84	9.8	16.4

Fick. Omitting this experiment the average difference between the two methods was only +0.2 per cent.

From the above evidence we conclude that the injection method as here described gives accurate figures for the cardiac output.

Experiments on man. Preliminary experiments were carried out on man and a few are presented here on account of their general interest. Our technique was slightly different from that reported previously (Hamilton et al., 1928a). Two hundred and fifty to five hundred milligrams of the dye in 2 cc. of distilled water (freshly prepared) were injected into the median basilic vein of an up-raised arm. Samples were taken from the femoral artery (Fraser et al., 1924; Binger, 1928) with the subject on his side at the edge of a stretcher. We used a 19 gauge stainless steel needle 7 cm. long, bent at right angles near the tip.

Table 2 summarizes the data of a few of these experiments on man.

SUMMARY

For the first time the accuracy of a method for calculating the cardiac output in man has been established by checking it in dogs against a reliable method, the direct Fick procedure. The results of a few human experiments are included.

BIBLIOGRAPHY

Binger, C. A. L. 1928. Journ. Clin. Invest., vi, 203.

Bock, J. and J. Buchscholtz. 1920. Arch. Exper. Path. u. Pharm., lxxxviii, 192.

Fraser, F. R., G. Graham and R. Hilton. 1924. Journ. Physiol., lviii, p. xxxiv.

Graham, E. A., W. H. Cole, G. H. Copher and S. Moore. 1926. Journ. Amer. Med. Assoc., lxxxvi, 467.

Gross, R. E. and R. Mittermaier. 1926. Pflüger's Arch., ccxii, 136.

Hamilton, W. F., J. W. Moore, J. M. Kinsman and R. G. Spurling. 1928a. This Journal, lxxxiv, 338.

1928b. This Journal, lxxxv, 377.

Henriques, V. 1913. Biochem. Zeitschr., lvi, 230.

1915. Ibid., lxxi, 481.

Henderson, Y. 1923. Physiol. Rev., iii, 165.

Himwich, H. E. 1928. Personal communication.

Kinsman, J. M., J. W. Moore and W. F. Hamilton. 1929. This Journal, lxxxix, 322.

Marshall, E. K., Jr. and A. Grollman. 1928. This Journal, lxxxvi, 117.

Stewart, G. N. 1897. Journ. Physiol., xxii, 159.

1921a. This Journal, lvii, 27.

1921b. This Journal, lviii, 20.

Van Slyke, D. D. and J. M. Neill. 1924. Journ. Biol. Chem., lxi, 523.

Wiggers, C. J. 1923. Circulation in health and disease. Lea & Febiger, Philadelphia.

Zuntz N. and Hagemann. 1898. Landwirtsch. Jahrbücher, suppl. iii, p. 371.

CONGENITAL PYLORIC OBSTRUCTION

I. Etiology: An Experimental Study

JESSIE LAIRD BRODIE[1]

From the Collins Laboratory of Nutritional Research, University of Oregon Medical School

Received for publication December 6, 1928

For a period of more than three years the Collins Laboratory in Nutritional Research has been investigating the problem of the effect upon the young of inadequate maternal diets during pregnancy and lactation, with special reference to inadequacy in the antineuritic vitamin. The tendency toward polyneuritis and hemorrhage has been detailed elsewhere (1) and in the present paper it is purposed to present a description of ten young which were born and nursed by mothers on a minimal vitamin B containing diet, and which presented a condition simulating that known in man as "congenital hypertrophic pyloric stenosis."

Osborne and Mendel (2) found in 1922 that 200 mgm. (or in our diets 2 per cent) of brewer's yeast was sufficient for the growth and reproduction of an albino rat. More recent investigations (3) tend to show that a female on this amount of the B factor cannot wean the majority of her young. They die during the nursing period with symptoms of polyneuritis, although the mother shows neither the characteristic clinical picture nor the autopsy findings of this disease.

The earliest manifestation of the deficiency in the young is the occurrence of hemorrhages in the skin, bones, muscles, brain, or other viscera with death of from 25 to 50 per cent of those in the litter (1), (3, B. Sure) (table 1). Then, as summarized in table 2, there may appear during the second week the condition which we have placed under the general term pyloric obstruction. A few of the young of the first generation and almost one-fourth of the young of the second generation develop marked emaciation, combined with a rapidly enlarging abdomen of extremely firm consistency. These animals, being rodents, cannot vomit. At autopsy, when the abdominal

[1] Presented to the Department of Surgery, May 15, 1928, in partial fulfilment of the degree of Doctor of Medicine, University of Oregon Medical School.

Appreciation is gratefully acknowledged to C. Ulysses Moore, Robert B. Hope, and Olaf Larsell for suggestions and technical assistance.

Read by invitation before the North Pacific Pediatric Society at Vancouver, B. C. June 28, 1928.

wall was slit, the stomach popped out as though under pressure. It was distended to at least three times its normal size by tightly packed curd which, on splitting the stomach wall, rolled out in a complete cast of the mucosa. It held its form perfectly until torn apart. The pylorus was tightly constricted, but patent to the extent that a small probe could be passed, although with difficulty. In some of the animals the pylorus appeared grossly hypertrophic. In every case in which the vagi were obtained and stained with osmic acid myelin degeneration was found. Several times hemorrhages were demonstrable in the muscles, abdominal viscera or brain.

Practically all of the young who live to the third week show clinically a marked paralysis, especially of the hind quarters, profuse watery stools and a subnormal temperature. The few who are strong enough to eat from the mother's ration or who are fed concentrated vitamin B by pipette,

TABLE 1

Manifestations of vitamin B deficiency in nursing albino rats

GENERATION	NUMBER OF FEMALES	NUMBER OF LITTERS	NUMBER OF YOUNG	NUMBER KILLED TO REDUCE LITTERS	NUMBER NURSED	NUMBER DYING IN FIRST WEEK OF HEMORRHAGE	NUMBER SHOWING PYLORIC OBSTRUCTION 2ND WEEK	NUMBER DYING OF POLYNEURITIS 3RD AND 4TH WEEKS	NUMBER WEANED
First.....	19	33	198	28	170	76 (45%)	2 (1.2%)	63 (38%)	32 (18%)
Second...	5	6	43	7	36	8 (22%)	8 (22%)	21 (58%)	2 (5.5%)

Maternal diet: Basal I, containing 2 per cent desiccated yeast.

Hemorrhage, pyloric obstruction, and polyneuritis form the outstanding pathology in the first, second, and third weeks, respectively. Some animals showed pyloric obstruction and later, polyneuritis.

usually make an uneventful recovery (1), (3, B. Sure). The majority, however, die. Autopsy is grossly negative except for occasional macroscopic visceral, subcutaneous, or intramuscular hemorrhages. Microscopically, however, the sciatic and other myelinated nerves show, when properly stained, degeneration of the myelin sheath.

With this complete history in mind, and noting that 65 to 90 per cent of those living to three weeks of age died from frank polyneuritis, a definite vitamin B deficiency disease, it does not seem inconsistent to consider the pyloric obstruction as merely another manifestation of the general deficiency of this vitamin. It is only upon the basal I diet[2] which is minimal in this factor that the condition has been found at all. Other diets identical, so far as we can tell, in all respects save for a larger amount of

[2] Basal I diet: 18 per cent casein, 3 per cent crisco, 2 per cent cod liver oil, 4 per cent salt mixture, (no. 185 McCollum), 71 per cent dextrin, and 2 per cent yeast (powdered yeast foam tablets of the Northwestern Yeast Company).

TABLE 2

Pyloric obstruction in albino rats nursed by mothers on a minimal vitamin B diet

FEMALE NUMBER	GENERATION	ORDER OF LITTER	NUMBER OF YOUNG NURSED	NUMBER SHOWING SYMPTOMS OF PYLORIC OBSTRUCTION	AGE WHEN SYMPTOMS FIRST NOTED	SEX	TREATMENT	OUTCOME OF THOSE	
								With stenosis	Without stenosis
					days				
647	2nd	1st	6	2	16	Not noted	None	1 killed (autopsy) 1 died (devoured)	4 died of polyneuritis
651	2nd	1st	6	2	14	Male	Atropine	1 died (devoured) 1 died (autopsied)	2 males died of polyneuritis Male and female weaned
651	2nd	2nd	5	4	7	Male	Atropine	1 died (devoured) 1 recovered (died later of polyneuritis) 2 died of polyneuritis superimposed on mild stenosis	1 killed (accident)
596	1st	2nd	6	2	6	Male Female	None	1 female killed and autopsied 1 male died and autopsied	4 females died of polyneuritis
Summary...........			23	10	6–16	87½% males		7 died of pyloric obstruction 3 died of polyneuritis	11 died of polyneuritis, 1 killed, 2 weaned

yeast, have never shown a single case of pyloric obstruction, nor have any of the hundreds of the litters on the stock diet.

Moreover it is interesting to note that pyloric obstruction developed in only 1.2 per cent of the young of the first generation raised on this diet restricted in vitamin B, while 22 per cent of the young of the second generation have shown the condition. It has long been known that vitamin B is not stored for any length of time in the animal organism (4) and when fed at a level minimal for growth any slight store would soon be depleted. The first generation is taken from the stock diet at the age of four to six weeks and their young show at least the terminal paralysis. The second generation never receive more than 2 per cent yeast, and when their young are born the deficiency must be extremely marked and begins to show earlier in the process of myelinogeny, e.g., when the vagi are being myelinated. If these escape sufficiently the peripheral nerves are the first to give indication of the disease. When two of the young with mild pyloric obstruction lived untreated to the age when myelination of the sciatics should have been completed the typical picture of polyneuritis was superimposed upon the primary condition.

One of the young of the first litter of female 651 (table 2) was given a dose of atropine sulphate comparable to that used for human babies but with entirely negative results. As this treatment was unsuccessful, the susceptibility of these animals to atropine was investigated both experimentally on normal animals and by consulting the literature. Sollmann (5) suggests that rats are very resistant to this drug, taking at least 2.5 grams per kilo as the minimal fatal dose. Our experimental findings showed an even greater resistance. In the later cases of pyloric obstruction, therefore, two of the males were given subcutaneous injections of 0.2 cc. of 0.1 per cent atropine sulphate for two consecutive days. The animal that had shown the most marked distention died and was devoured on the second day. The other male showed a very soft stomach so the dose was reduced to 0.1 cc. on the third day and then discontinued. In a few days he developed a marked diarrhea and paralysis and died of polyneuritis.

Many of our attempts to secure the vagus in these animals were unsuccessful. In the first cases we did not realize the significance of obtaining this nerve; in many of the later ones the mother devoured at least a large portion of the body; and in a few animals the specimens dissected from these five-gram animals showed themselves to be blood vessels or other tissues when we thought we had the thread-like vagus. Older animals, three to four weeks, nursed by mothers on this same diet and dying with symptoms of polyneuritis practically always showed some degeneration of the vagus, although never to the marked extent of that found in the sciatic. In these cases the only symptoms referable to the gastro-intestinal

tract were loss of appetite and a terminal diarrhea, and at autopsy gaseous distention and atony of the bowel. When pyloric obstruction was most marked (young of female 596) there was an extensive degeneration of the vagus comparable to the extreme gastro-intestinal symptoms. Further work along this line is certainly needed before the exact relationship of the degeneration of the vagus to the pyloric obstruction is ascertained.

Studies of the literature show us also a dearth of knowledge on three other questions intimately associated with this problem: 1, the function of the myelin sheath; 2, the significance of myelin degeneration in the clinical picture of vitamin B deficiency; and 3, the nervous control of the pyloric sphincter.

Fig. 1. Litter mates from mother on minimal B diet. Animal on left shows emaciation, stunting of growth, and distention of the abdomen. At autopsy the latter was found due to gastric dilatation secondary to pyloric obstruction. The brother on the right has normal growth and average abdominal size.

The myelin sheath is believed (6) to serve the function either of insulation or nutrition to the nerve, and consequently a disease affecting this material may have two stages, an early period in which there is irritability and a later one where the nerve is entirely non-functional. Clinically beri-beri shows these two stages.

Work is now being carried on in this laboratory to find out if possible how a deficiency of the anti-neuritic vitamin affects myelinogeny or nerve development in general. Babies show a vitamin B deficiency on diets minimal in this factor when their mothers show no symptoms of the disease. Is it due to a high mammary threshold for the anti-neuritic factor, or does the developing nervous system require a larger amount of this "metabolic

building stone" (7)? The vagus is not fully formed at the time of birth in any species, but varies from practically no myelin formation in the rat (8) to a very small number of myelinated fibers in the higher species of mammals (9). Myelination takes place very rapidly after birth. Many workers on this subject hold that myelin degeneration is merely the indicator of a more extensive process taking place in the nerve cells proper. Vedder (7) claims that the antineuritic vitamin is a building stone which is essential for the normal metabolism of nervous tissue. "A certain amount of this vitamin is constantly necessary in order to maintain the nervous system in healthy condition. If the supply of vitamin is cut down by any dietary that contains an insufficient amount of this substance the normal metabolism of the nervous system at once suffers. If the faulty diet is continued, degeneration of the nervous system progresses steadily until a point is reached at which the symptoms of beri-beri appear." Ellis (10) finds from his more complete histological studies that the sympathetic unmyelinated fibers are also widely involved.

The nervous control of the pyloric sphincter cannot be divided as can that of other parts of the gastro-intestinal tract into sympathetics and parasympathetics that are directly antagonistic (11). Both the splanchnics and vagi of this region are mainly motor in function, although containing inhibitor as well as motor fibers. The tonus of the musculature determines the end result of the stimulus (12).

Some of the recent work (13) indicates that in pyloric obstruction we have overstimulation of the motor nerves, while others seem to show (14) that lack of stimulation causes contraction rings. Much more work must be done before a definite conclusion can be reached as to whether the pyloric obstruction is due to an early degeneration of the vagus resulting in overstimulation of the pyloric antrum or perhaps lack of stimulation over the parasympathetics in the late stages resulting in contraction rings. Against this latter theory, however, are the well known beneficial results in pylorospasm of babies from the use of atropine, which paralyzes the parasympathetic nerve endings.

It may be difficult to see how a comparison can be made between a condition in rats in which the stomach becomes markedly distended with food and one in human babies in which food can not be retained in the stomach. But we must bear in mind the fact that we are dealing in the first instance with rodents, animals that are incapable of vomiting (15). As this repeated taking of food into the stomach continues, with little or no discharge through the pylorus, the strenuous action of the pyloric antrum results in a firm packing of the milk curd into the cast found at autopsy. In the human baby the strenuous and ineffectual peristalsis produces a marked projectile type of vomiting.

It has been found, in this and other laboratories where experiments on

the production of polyneuritis in young animals are going on, that on the whole the males fall sick earlier than the females and show a more severe form of the disease. In Japan, the Philippines and other countries where it is so prevalent, beri-beri is reported to attack males much oftener than females (16). In our experimental cases of pyloric obstruction the sex of one litter was not recorded, but out of the other eight animals showing the condition seven or 87.5 per cent were males (table 2). Clinical reports of congenital hypertrophic pyloric stenosis all agree that a preponderance of cases is found among boy babies. Many authors mention as high as 90 per cent incidence in males with a mortality two or three times that of females (17).

It is unnecessary to go into detail at this time in regard to existing theories concerning etiology (18). The arguments have waged for years as to whether the hypertrophy preceded the spasm or the spasm caused the hypertrophy, but all attempts at proof both experimentally and clinically have ended unsuccessfully, so far as we have been able to ascertain. In respect to the tumor formation our experiments are also inconclusive. The stomachs of the young rats were so distended and stretched with food that it was difficult to make a satisfactory comparison, although we felt that in many cases a gross hypertrophy could be made out. Sections were not satisfactory, for we found, in agreement with Hertz, Mohr, Aschoff, Pfaundler (19) and others, that the plane of section, hardening fluids, size of sections and stage of contraction all must be taken into consideration.

Thomson (20) and Haas (21) have been strongest in the support of the theory that hypertrophic pyloric stenosis is merely an advanced degree of pylorospasm due to a functional disturbance of the nerves of the stomach and pylorus leading to ill coördinated and therefore antagonistic action. But no one has presented a reason for this "disturbance of the physiological function of the vegetative nervous system." In the experimental work presented above the condition was associated with a minimal amount of the anti-neuritic vitamin.

Some very interesting and conclusive work has recently been published by Macy and associates (22) from the Nutritional Research Laboratories of the Merrill-Palmer School and Children's Hospital of Michigan. They find that the antineuritic potency of the mixed milk of a group of wet nurses receiving the average American dietary is very slight. "In view of the facts presented, it is possible that many mothers do not supply enough of vitamin B to their babies."

Much more work is necessary along the line of both the experimental production of the disease in animals and the checking of maternal dietary histories where the disease occurs in humans. Only when this is coupled with numerous autopsy reports of babies dying of pyloric stenosis, including a study of nerves, can we reach a definite decision as to the probability of a vitamin deficiency as the basis of certain cases of human pyloric obstruction.

SUMMARY

1. Protocols are presented of four litters of albino rats in which ten out of twenty-three young showed pyloric obstruction. The diet of the mother was minimal in the anti-neuritic vitamin.

2. The clinical picture presented was an enlarged and very firm abdomen with extreme general emaciation. At autopsy the stomach was found enlarged and packed with curd, and the vagus in all cases examined showed myelin degeneration.

3. In the cases in which the condition was mild or cured by atropine, polyneuritis was later superimposed upon the original condition. The pyloric obstruction is therefore considered as one manifestation of vitamin B deficiency with resulting defective myelinogeny.

4. One and two-tenths per cent of the young of the first generation showed this condition, and twenty-two per cent of the young of the second generation.

5. Eighty-seven and a half per cent of the young thus affected were males.

6. Comparison is made between this condition in rodents, who cannot vomit, and human babies with congenital pyloric stenosis. The age of onset, sex, and response to atropine all point to a similarity between the two conditions.

BIBLIOGRAPHY

(1) Moore, C. U., J. L. Brodie and R. B. Hope. 1927. This Journal, lxxxii, 350.
Moore, C. U. and J. L. Brodie. 1927. Amer. Journ. Dis. Child., xxxiv, 53.
Manville, I. A., J. L. Brodie and C. U. Moore. 1926. Northwest Med., xxv, 205.

(2) Osborne, R. B. and L. B. Mendel. 1922. Journ. Biol. Chem., liv, 739.

(3) Anderegg, L. T. 1924. Journ. Biol. Chem., lix, 587.
Evans, H. M. and H. S. Burr. 1928. Journ. Biol. Chem., lxxvi, 263.
Grant, A. H. 1924. Univ. Cincinnati Med. Bull., iii, 17.
Hartwell, G. A. 1921. Biochem. Journ., xv, 140, 563; 1925, Ibid., xix, 1075.
Heller, V. G. 1923. Journ. Biol. Chem., lv, 385.
Hogan, G. A. and H. M. Harshaw. 1924. Journ. Met. Res., v, 3.
Manville, I. A. 1926. Science, lxiv, 256.
Mattill, H. A. and N. C. Stone. 1923. 'Journ. Biol. Chem., lv, 443.
Nelson, P. M. 1926. Journ. Home Econ., xviii, 7.
Nelson, V. E., R. L. Jones, V. G. Heller, T. B. Parks and E. I. Fulmer. 1926. This Journal, lxxvi, 325.
Sure, B. 1927. Journ. Amer. Med. Assoc., lxxxix, 675; Journ. Biol. Chem., lxxiv, 55; Proc. Amer. Soc. Biol. Chem., Idem., lxxiv, 681.
Sure, B. and S. J. Schilling. 1928. Amer. Journ. Dis. Child., xxxv, 811.

(4) Steenbock, H., M. T. Sell and J. H. Jones. 1923. Journ. Biol. Chem., lv, 411.

(5) Sollmann, T. 1917. W. B. Saunders Co. Appendix H, p. 323.

(6) Starling, E. H. 1926. Philadelphia, Lea & Febiger.

(7) Vedder, E. B. 1913. Wood & Co., N. Y.
Vedder, E. B. and F. Clark. 1921. Philippine Journ. Sci., vii.
(8) Cornwall, L. H. and R. M. Brickner. 1928. Unpublished data.
Cornwall, L. H. 1927. Arch. Neurol. and Psychiat., xviii, 240.
(9) Feldman, W. M. 1920. Longmans, Green & Co., London.
(10) Ellis, W. G. 1898. Lancet, ii, 985.
(11) Chase, M. R. 1916. Journ. Comp. Neurol., xxvi, 421.
Hammett, F. S. 1921. This Journal, lv, 414.
McCrea, E. D. and B. A. McSwiney. 1926. Journ. Physiol., lxi, 28.
Thomas, J. E. and A. Kuntz. 1926. This Journal, lxxvi, 598.
(12) McCrea, E. D., B. A. McSwiney and J. S. B. Stopford. 1925. Quart. Journ. Exper. Physiol., xv, 200.
(13) Carlson, A. J. and S. Litt. 1924. Arch. Int. Med., xxxiii, 281.
McCrea, E. D., B. A. McSwiney and J. S. B. Stopford. 1926. Quart. Journ. Exper. Physiol., xvi, 195.
Thomas, J. E. and H. Wheelon 1922. Journ. Lab. and Clin. Med., vii, 7.
(14) Alvarez, W. C. 1928. Paul B. Hoeber.
Cannon, W. A. 1908. This Journal, xviii, 429.
(15) Hatcher, R. A. and S. Weiss. 1922. Arch. Int. Med., xxix, 690.
Hatcher, R. A. 1924. Physiol. Rev., iv, 479.
(16) Takano, R. 1926. Japan Med. World, vi, 8.
(17) Alexander, E. G. 1924. Med. Clinics of N. A., viii, 838.
Richter, H. M. Abt's Pediatrics, iii, 452.
Davison, W. C. 1925. Bull. Johns Hopkins Hosp., xxxvii, 75.
Goldbloom, A. 1920. Amer. Journ. Dis. Child., xix, 263.
Heusch, K. 1922. Zeitschr. f. Kinderh., xxxi, 158.
(18) Sauer, L. W. 1924. Arch. Ped., xli, 145.
Moore, H. L. 1924. South. Med. Journ., xvii, 187.
Davis, H. H. 1924. Journ. Amer. Med. Assoc., lxxxiii, 686.
Bilberback, J. B. 1928. Northwest Med., xxvii, 182.
(19) Aschoff, L. 1919. Jena. "Pathologische Anatomie", 788.
Hertz. 1916. Jahrb. f. Kinderh., lxxxii, 32, 131.
Mohr, M. 1921. Zeitschr. f. Kinderh., xxii, iii.
Pfaundler, M. 1909. Jahrb. f. Kinderh., lxx, 253.
(20) Thomson, J. 1896. Edinb. Hosp. Re. Edinb. and Lon. iv, 16.
(21) Haas, S. V. 1918. Amer. Journ. Dis. Child., xv, 323; 1919. Arch. Ped., xxxvi, 516; 1922. Journ. Amer. Med. Assoc., lxxix, 1314.
(22) Macy, I. C., J. Outhouse, A. Graham and M. L. Long. 1927. Journ. Biol. Chem., lxxiii, 189.

STUDIES ON SUPRARENAL INSUFFICIENCY

V. The Non-protein Nitrogen and Urea in the Blood of Suprarenalectomized Rats

LELAND C. WYMAN and BURNHAM S. WALKER

From the Physiological Laboratory of Boston University School of Medicine and the Evans Memorial, No. 200

Received for publication April 13, 1929

It has frequently been shown that in cases of Addison's disease and in suprarenalectomized animals the non-protein nitrogen and the urea of the blood are increased. In over half of the cases of adrenal failure studied in the clinics of the Evans Memorial the non-protein nitrogen was found to be higher than normal, and in nearly a fifth of the cases the urea nitrogen was higher than normal (Rowe, 1928). Rowntree (1925) found increased blood urea in several of his cases of Addison's disease.

Marshall and Davis (1916) found in suprarenalectomized cats a rise in the blood urea to about twice the normal value, with a further rise shortly before death. They also found that these animals excreted much less urea and creatinine in the urine after an injection of these substances than their control cats, and suggested that the suprarenals may secrete some substance which is necessary for the maintenance of normal kidney function. Bevier and Shevky (1919) observed that suprarenalectomy in rabbits was followed by a depression of the rate of urea excretion by the kidneys, and attributed this fact to lack of medullary secretion. They postulated a control of urea excretion by an epinephrin-pituitrin balance. Banting and Gairns (1926) and Lucas (1926) found an increase in non-protein nitrogen and urea up to three or four times the normal amount as a terminal stage phenomenon in suprarenalectomized dogs. They suggested that this may be the result of kidney injury, since they found nephrosis at autopsy. Rogoff and Stewart (1926) noted in suprarenalectomized dogs a marked increase in non-protein nitrogen and in urea coincident with the appearance of characteristic symptoms and becoming greater as time went on up to death. Hartman, MacArthur, Gunn, Hartman and MacDonald (1927) found in suprarenalectomized cats, and in cats and a rabbit with "transient suprarenal insufficieny," a marked increase in blood urea which paralleled the severity of the symptoms of insufficiency. They also observed an accumulation of large quantities of lipoid substances in the cortex

of the kidney. Swingle (1927) found in suprarenalectomized cats a progressive increase in non-protein nitrogen and urea, beginning within twenty-four hours after operation. He described slight pathological changes in the kidneys.

Gunn, Cori and Hartman (1928) described lipoid nephrosis in guinea pigs and rats which had died of acute suprarenal insufficiency. Marine (1928) reported acute tubular nephritis or lipoid nephrosis in suprarenalectomized cats, dogs, rabbits and rats. He stated that cats show the most striking changes, that the fatty metamorphosis is much milder in rabbits and rats, and suggested that this may be related to the longer period of survival of these latter animals. Recently, Zwemer and MacMahon (1929) have described the appearance of fairly large quantities of fat in the convoluted tubules of suprarenalectomized cats, and stated that the amount of fat is correlated with the survival time.

This paper reports the results of a study of the non-protein nitrogen and urea in the blood of suprarenalectomized rats,—animals which may live for a comparatively long time after double suprarenalectomy, exhibiting meanwhile a chronic type of insufficiency. An adequate account of the non-protein nitrogen and urea in the blood of animals which show chronic as well as acute suprarenal insufficiency after suprarenalectomy was not found in the literature.

Methods. A mixed stock of colored varieties of the albino rat was used. The selection of animals, care of the animal room, diet, post-operative care, technique of suprarenalectomy, of autoplastic cortical transplantation and of control blank operations, microscopic appearance of transplants, and the terminology indicating the degree of insufficiency have been described in previous papers from this laboratory (see Wyman and Walker, 1929). Approximately equal numbers of each sex were used and no sex differences with respect to the blood findings were observed. Adult rats from five to twelve months old were selected for suprarenalectomy. The younger rats were used in experiments in which blood was taken for examination at relatively long periods after operation so that blood was studied in all cases in rats from seven to twelve months of age. Autoplastic cortical transplants were made in rats from one and a half to four months old and the blood was studied when they were from seven to nine months of age. All transplants and suspected cortical accessories were fixed in Müller's fluid, sectioned and examined microscopically.

Blood was taken for non-protein nitrogen and urea nitrogen determinations by lightly etherizing the rat and exsanguinating from the heart, receiving the blood directly in a test tube containing 20 mgm. potassium oxalate. Determination of urea and non-protein nitrogen was performed by the Folin-Wu method.

Normal rats. In a series of nine healthy, unoperated rats about nine

months of age the non-protein nitrogen of the blood varied from 35 to 42 mgm. per 100 cc., averaging 38 mgm. The urea nitrogen varied from 19 to 25 mgm. per 100 cc., averaging 23 mgm. (table 1).

Suprarenalectomy. In a series of eighteen doubly suprarenalectomized rats determinations of the urea and non-protein nitrogen of the blood were made from 85 to 234 days after operation (table 2). The analytical findings separated these rats into two groups, those with normal urea and non-protein nitrogen values, and those with increased values. These groups were also characterized by the presence or absence of gross accessory cortical tissue. In nine rats with gross accessory cortical tissue the non-protein nitrogen varied from 32 to 45 mgm. per 100 cc., averaging 38 mgm. The urea nitrogen varied from 12 to 25 mgm. per 100 cc., averaging 18 mgm. These rats were healthy, had abundant abdominal fat, and had gained from 33 to 104 grams since operation.

In nine rats having no demonstrable accessory cortical tissue the non-protein nitrogen varied from 47 to 68 mgm. per 100 cc., averaging 57 mgm. The urea nitrogen varied from 29 to 46 mgm. per 100 cc., averaging 38 mgm. With one exception, these rats failed to show a gain in weight comparable with that of the other group. They were characterized by enlarged thymus and lymph glands and little or no abdominal fat. These rats, while alive, had no outstanding symptoms of insufficiency, and on the basis of autopsy findings may be classed as chronically insufficient.

TABLE 1

Normal unoperated rats

	MGM. PER 100 CC. WHOLE BLOOD									AVERAGE
N.P.N.	35	36	37	38	38	38	38	41	42	38
Urea-N	19	23	23	19	23	23	24	25	25	23

In another series of 30 rats, the determinations were made at a shorter interval after operation, from 10 to 32 days (table 3). Gross accessory cortical material was found in only three cases. In two of these (nos. 50 and 56) the rats had gained weight, showed no symptoms of insufficiency, and the blood analytical findings showed no marked variations from the normal. In the other case (no. 70), although the rat had gained weight, she had slight symptoms of insufficiency (bloody eyes, diarrhea) and the non-protein nitrogen was somewhat high. The cortical accessory in this case was small (1 mm. diameter). All the other rats in this series had lost weight since operation (4 to 42 grams) with the exception of one which was pregnant. These rats fell into three general groups according to symptoms. Nine had no external symptoms of suprarenal insufficiency.

TABLE 2

Suprarenalectomized rats (long period)

RAT NUMBER	DAYS AFTER OPERATION	N.P.N.	UREA-N.	CORTICAL ACCESSORIES
		mgm. per 100 cc.	*mgm. per 100 cc.*	
12	140	32	12	CA
9	234	33	20	CA
57	148	35	16	CA
16	140	35	16	CA
11	140	38	17	CA
100	149	40	12	CA
19	140	41	25	CA
110	149	45	19	CA
8	104	45	24	CA
Average...		38	18	CA
18	87	47	30	O
7	104	49	29	O
5	104	55	34	O
10	103	56	39	O
6	104	57	39	O
14	97	59	45	O
13	98	61	37	O
15	97	62	46	O
3	85	68	44	O
Average...		57	38	O

TABLE 3

Suprarenalectomized rats (short period)

RAT NUMBER	DAYS AFTER OPERATION	N.P.N.	UREA-N.	SYMPTOMS OF INSUFFICIENCY	CORTICAL ACCESSORIES
		mgm. per 100 cc.	*mgm. per 100 cc.*		
50	20	37	26	None	CA
58	32	43	27	None	O
56	18	44	28	None	CA
46	20	51	39	None	O
70	30	52	27	Slight	CA (small)
62	24	53	34	None	O
68	24	55		None	O
52	11	60	44	Moderate	O
37	13	63	39	Moderate	O
59	22	63		None	O
41	16	65	39	None	O
69	24	66		None	O
43	14	71	46	None	O
65	21	73		Moderate	O
34	17	74	68	None	O
21	16	77		Severe	O
61	17	78	55	Severe	O
49	14	81	52	Moderate	O
47	20	84		Moderate	O
66	21	86		Moderate	O
39	13	88	53	Moderate	O
48	13	88		Moderate	O
40	13	92	40	Moderate	O
51	10	94	50	Moderate	O
71	24	96		Moderate	O
38	17	102	73	Moderate	O
53	20	104	78	Moderate	O
54	10	105	76	Severe	O
45	20	107		Severe	O
55	14	145	106	Terminal	O
Average...		57	38	None	
		83	51	Moderate or slight	
		102	79	Severe or terminal	

Thirteen had moderate symptoms varying in degree, such as asthenia, sluggishness, bloody eyes and nose, emaciation, diarrhea. Five had the above symptoms in more severe form, together with evidences of lowered temperature. In one case (no. 55) the blood was taken during

the terminal convulsive stage. The autopsy findings in all cases were consistent with the symptoms observed before death. These three groups may possibly include rats with all degrees of suprarenal insufficiency, acute, subacute and chronic. An inspection of the table shows that the non-protein nitrogen and urea tend to increase as the symptoms of insufficiency become more severe. This is well demonstrated by the three sets of average values given in table 3.

Control operation. In a series of nine rats determinations of the non-protein nitrogen and urea of the blood were made from 13 to 26 days after a control operation consisting of exposure of both suprarenal glands and the removal of a piece of fat from near each suprarenal site. These rats

TABLE 4

Control rats (blank operated)

RAT NUMBER	DAYS AFTER OPERATION	N.P.N.	UREA-N.
		mgm. per 100 cc.	*mgm. per 100 cc.*
C4	26	28	13
C3	26	30	17
C2	26	32	17
C10	13	37	
C24	16	38	
C9	13	40	20
C1	19	43	21
C8	13	54	35
C6	13	57	31
Average........		40	22

TABLE 5

Rats with autoplastic cortical transplants

RAT NUMBER	DAYS AFTER OPERATION	N.P.N.	UREA-N.	CORTICAL ACCESSORIES
		mgm. per 100 cc.	*mgm. per 100 cc.*	
T7	68	29	20	CA
T14	156	29	21	CA
T62	147	36	16	O
T71	145	38	18	O
T46	234	38	19	O
T13	156	38	23	CA
T17	155	39	23	O
T47	234	41	20	O
T15	156	41	23	O
T67	145	42	20	O
T58	148	46	25	O
T61	230	49	27	O
Average......		39	21	

were about eleven months of age. The non-protein nitrogen varied from 28 to 57 mgm. per 100 cc., averaging 40 mgm. The urea nitrogen varied from 13 to 35 mgm. per 100 cc., averaging 22 mgm. (table 4). Inspection of the table shows that moderately high values for both substances may occasionally be obtained during the first two weeks after such an operation. This is not surprising since the field of operation is near the kidney and may involve some temporary disturbance of that organ. These rats remained in good health after the operation and at autopsy abundant abdominal fat was found and the suprarenal glands appeared normal.

Autoplastic cortical transplantation. Determinations of the non-protein nitrogen and urea of the blood were made on twelve rats from 68 to 234 days

after autoplastic transplantation of both suprarenal glands. The non-protein nitrogen varied from 29 to 49 mgm. per 100 cc., averaging 39 mgm. The urea nitrogen varied from 16 to 27 mgm. per 100 cc., averaging 21 mgm. (table 5). The range of these values superimposes upon the normal range, and the average values agree with the normal average values. At autopsy from one to three well vascularized masses of cortical tissue were found at the sites of transplantation. These masses were from 1 to 5 mm. in diameter. Upon microscopic examination they appeared to be composed of healthy cortical tissue. No chromaffin tissue could be identified. In three of these cases a gross accessory mass of cortical tissue about 5 mm. in diameter was found near one of the suprarenal sites. In all cases the rats were fat and healthy and had gained considerable weight since operation. Evidently chromaffin tissue is not essential for the maintenance of normal levels of blood non-protein nitrogen and urea.

Discussion. The results reported in this paper agree with the observations of other workers who have investigated the non-protein nitrogen and urea of the blood of other animals in suprarenal insufficiency, in that the amount of increase of these substances in the blood of rats after suprarenalectomy appears to be correlated with the severity of suprarenal insufficiency, the greatest increases being observed in the terminal stages. The authors are not prepared to say whether or not the increases of non-protein nitrogen and urea in the blood of suprarenalectomized rats are consequent to kidney injury. The results, however, are consistent with the observation of Marine that fatty metamorphosis of the kidney is much milder in rats than in animals which die sooner after suprarenalectomy. It is also significant that Zwemer and MacMahon found that the amount of fat in the kidneys of suprarenalectomized cats is correlated with the survival time. Whatever the cause of the increases in non-protein nitrogen and urea of the blood, it is dependent upon a loss of cortical function rather than of medullary function. This is in harmony with the suggestions of various authors that some influence from the suprarenal cortex, possibly a secretion, is concerned in the maintenance of normal kidney function.

Much of the recent information concerning the physiology of the suprarenal cortex points towards a steady maintenance of certain bodily conditions by some influence from that organ. By a steady maintenance it is implied that the influence from the cortex is constantly acting, possibly requiring appreciable time to produce its effects, and is not a factor which lies in reserve ready to be called upon. Suprarenalectomy would remove this influence and as a result the maintained conditions would be disturbed. The action of the other part of the gland, the suprarenal medulla, in supplying influences to readjust certain bodily equilibria when called upon in emergency conditions is well known.

SUMMARY

1. Following double suprarenalectomy in rats the non-protein and urea nitrogen of the blood may be increased, the amount of increase tending to parallel the severity of the symptoms of suprarenal insufficiency. Such increases were not noted in rats having either gross accessory cortical tissue or successful cortical transplants, in the absence of demonstrable chromaffin tissue.

2. Following control blank operation infrequent, moderately high values for both substances may be found during the first two weeks after operation, but these values are not as high as those observed in suprarenal insufficiency. Otherwise the values obtained are normal.

3. The results indicate that the high values for non-protein and urea nitrogen of the blood observed after suprarenalectomy are associated with cortical insufficiency. It is suggested that this is further evidence for a function of the suprarenal cortex concerned with the steady maintenance of certain bodily conditions.

The authors wish to express their indebtedness to Miss Caroline tum Suden for technical assistance.

BIBLIOGRAPHY

Banting, F. G. and S. Gairns. 1926. This Journal, lxxvii, 100.
Bevier, G. and A. E. Shevky. 1919. This Journal, l, 191.
Gunn, F. D., C. F. Cori and F. A. Hartman. 1928. Proc. Soc. Exper. Biol. and Med., xxv, 410.
Hartman, F. A., C. G. MacArthur, F. D. Gunn, W. E. Hartman and J. J. MacDonald. 1927. This Journal, lxxxi, 244.
Lucas, G. H. W. 1926. This Journal, lxxvii, 114.
Marine, D. 1928. Arch. Pathol., vi, 169.
Marshall, E. K. and D. M. Davis. 1916. Journ. Pharm. Exper. Therap., viii, 525.
Rogoff, J. M. and G. N. Stewart. 1926. This Journal, lxxviii, 711.
Rowe, A. W. 1928. Endocrinol., xii, 1.
Rowntree, L. G. 1925. Journ. Amer. Med. Assoc., lxxxiv, 327.
Swingle, W. W. 1927. This Journal, lxxix, 666.
Wyman, L. C. and B. S. Walker. 1929. This Journal, in press.
Zwemer, R. L. and H. E. MacMahon. 1929. Anat. Record, xlii, 43

STUDIES ON SUPRARENAL INSUFFICIENCY

VI. Anaphylaxis in Suprarenalectomized Rats

LELAND C. WYMAN

From the Physiological Laboratory of Boston University School of Medicine and the Evans Memorial, No. 201

Received for publication April 22, 1929

In a previous paper in this series it was reported that the increased susceptibility to histamine of suprarenalectomized rats is largely due to the lack of medullary tissue, rather than to the lack of cortical tissue (Wyman, 1928). The remarkable resemblance between histamine reactions and anaphylactic phenomena has been pointed out by numerous investigators and has led to the theory that a fundamental factor in the anaphylactic reaction is the liberation of histamine or substances having a histamine-like action from sensitized cells (see Lewis, 1927, p. 112). It seemed pertinent, therefore, to investigate anaphylaxis in suprarenalectomized rats in the same manner in which the susceptibility to histamine was previously dealt with.

Parker and Parker (1924) have shown that anaphylactic shock can be produced in the white rat after both active and passive sensitization. Flashman (1925) reported that suprarenalectomized rats show increased susceptibility to anaphylactic shock and concluded that this effect is related to the degree of suprarenal insufficiency. This paper came under notice while the present study was in progress, and after a careful inspection of the contents it was felt that the conclusions drawn were based on data which were far from satisfying.

Methods. A mixed stock of colored varieties of the albino rat was used. The details concerning care of the animals, technique and results of various operations, microscopical examination of suprarenal tissue, and the terminology used have been given in previous papers (see Wyman and Walker, 1929). Adult rats from five to twelve months old were selected for the experiments. The younger rats were used in experiments in which sensitization was carried out at relatively long periods after operation. Autoplastic cortical transplants were made in rats from one and a half to three months old and sensitization was carried out when they were from five to nine months of age. No sex differences with respect to the experiments in hand were noted.

Normal horse serum, containing no preservative, was obtained from the

Massachusetts Department of Public Health (lot nos. 51 and N-54-B). The rats were sensitized by giving three intraperitoneal injections of 1 cc. of horse serum at two day intervals. The test dose of 1 cc. of horse serum was given by intraperitoneal injection on the tenth, eleventh or twelfth day after the last sensitizing dose. In the first few cases (six) sensitizing and test doses of 1 cc. per 100 grams of body weight were used, but since these animals showed no differences in reaction from those in which doses of 1 cc. were used the first method was abandoned. Careful observation after each sensitizing dose disclosed no untoward reactions, indicating that the horse serum used had no natural toxicity for the rat. Complete autopsies were performed on all rats.

Suprarenalectomy. Twenty-five doubly suprarenalectomized rats were sensitized after operation by injections of horse serum. In twelve cases the first sensitizing dose was given from twelve to sixteen days after suprarenalectomy. The test dose was given from twenty-three to twenty-seven days after operation. Following the test dose five had slight symptoms, two had moderate symptoms, three had severe symptoms (two of these died about twelve hours after injection), and two died 60 and 147 minutes after injection. The survivors were killed for autopsy four or five days later. With the exception of one rat which had moderate symptoms and had lost 4 grams, all these rats had gained weight since operation (8 to 42 grams). Varying amounts of abdominal fat were found at autopsy, but more than half had little or no abdominal fat. All had enlarged thymus and lymph glands. In three cases, one with slight and two with severe symptoms, a small mass (1 to 1.5 mm. diameter) of what appeared in section to be regenerating cortical tissue was found near one suprarenal site. This group might possibly include rats with subacute and chronic suprarenal insufficiency, as well as those destined to become normal with respect to cortical function through the regeneration of accessory tissue.

In thirteen other cases the first sensitizing dose was given from 85 to 168 days after suprarenalectomy and the test dose was given ten or eleven days later. Of these rats three had slight symptoms, one had moderate symptoms, two had severe symptoms, and seven died from 30 to 143 minutes after injection. The survivors were killed for autopsy five or seven days later. At autopsy eight of the thirteen were found to have gross accessory cortical masses from 2 to 8 mm. in diameter, in or near the suprarenal sites. Two rats had two such masses. Microscopic examination showed these to be well vascularized bodies composed of healthy appearing cortical cells. The amount of cortical tissue present in most cases was fully as much as that present in two normal suprarenal glands. No signs of accessory chromaffin tissue could be found. These eight rats were fat and healthy and had gained weight since operation

(27 to 163 grams). Of these eight cases four had died, two had severe symptoms, one had moderate symptoms, and one had slight symptoms. In the other five cases, three of which had died, no gross accessory suprarenal tissue was found. These rats appeared to be healthy and had gained weight since operation (59 to 95 grams). Little or no abdominal fat, however, was found at autopsy, and in all the thymus and lymph glands were enlarged. In the absence of gross accessory tissue they may be classed as chronically insufficient.

One rat in this group deserves special mention because she showed a reaction which is rarely seen in laboratory animals. She was one which had slight symptoms and was found at autopsy to have a gross accessory cortical mass. Following the test dose of horse serum a marked edema of the entire face and of the fore-feet appeared which lasted for about twelve hours. The face was so swollen that the eyes were nearly closed and the lips were especially swollen so that the muzzle appeared very broad. Four days later another test dose of horse serum was given and the same reaction appeared again, but to a less degree and was less prolonged.

Normal rats and control operation. Twenty-nine normal rats were sensitized by injections of horse serum and the test doses were given eleven or twelve days later. Following the test dose ten had no symptoms of anaphylactic shock, thirteen had slight symptoms, and six had moderate symptoms. The results differed from those of Parker and Parker in that none of the rats died or even had severe symptoms. In two other rats a control operation was done, consisting of removal of one suprarenal gland and exposure and handling of the other. In these rats the first sensitizing dose was given eleven and thirteen days after operation and the test dose was given eleven days later. Both had slight symptoms following the test dose. Comparison of these results with those obtained in suprarenalectomized rats shows an increased susceptibility to anaphylactic shock following double suprarenalectomy.

Suprarenalectomy after sensitization. Fifteen of the sensitized rats described above were suprarenalectomized on the day following the injection of the test dose of horse serum. On the seventh, eighth or ninth day after operation a second test dose of horse serum was injected. Following this procedure one had slight symptoms, one had moderate symptoms, one had severe symptoms (died about twelve hours after injection), and twelve died from 40 to 210 minutes after injection. These rats had had no marked change in weight since operation and at autopsy varying amounts of abdominal fat were found. No gross accessory suprarenal tissue was found. The great mortality in this group is not surprising in view of the fact that the second test doses were given so soon after operation. As a number of investigators have repeatedly pointed out, experiments done on animals too soon after suprarenalectomy, before they have

had sufficient time to recover from the trauma of operation, are liable to misinterpretation. It is believed, however, that the results do show that the increased susceptibility after suprarenalectomy is dependent upon loss of the glands, and is not consequent to some peculiarity of sensitization in the absence of the suprarenals.

Autoplastic cortical transplantation. Fifteen doubly suprarenalectomized rats, having autoplastic cortical transplants in the abdominal muscles, were sensitized by injections of horse serum. The first sensitizing dose was given from 83 to 171 days after operation and the test dose was given ten or eleven days later. Following the test dose seven had slight symptoms, one had moderate symptoms, one had severe symptoms (died about twelve hours after injection), and six died from 41 to 279 minutes after injection. The survivors were killed for autopsy seven days later. All these rats were fat and healthy and had gained considerable weight since operation (53 to 156 grams). At autopsy from one to three well vascularized masses of cortical tissue were found at the sites of transplantation. These masses were from $1\frac{1}{2}$ to 7 mm. in diameter. Microscopic examination showed them to be composed of healthy appearing cortical tissue. Five of these rats had, in addition, a gross accessory mass of cortical tissue from $1\frac{1}{2}$ to 4 mm. in diameter near one suprarenal site. A number of these rats appeared to have, therefore, more cortical tissue than is present in a normal animal. Comparison of the mortality in this group, 40 per cent, with that in the group of suprarenalectomized rats, 36 per cent, shows that increased susceptibility to anaphylactic shock following suprarenalectomy is not dependent upon cortical insufficiency. It may be inferred, therefore, that the increased susceptibility is dependent upon the lack of medullary tissue. This is consistent with the well known use of adrenalin in alleviating the symptoms of serum sickness in human beings.

Adrenalin injections. An attempt to reduce the susceptibility to anaphylactic shock after suprarenalectomy by means of injections of adrenalin chloride solution was not entirely successful. Such an attempt was made in sixteen cases. Ten of these rats were sensitized nineteen or twenty days after suprarenalectomy and six were sensitized thirteen days before suprarenalectomy. Intraperitoneal injections of 1 cc. of adrenalin chloride solution (Parke, Davis & Company), freshly diluted in sterile saline solution to 1:10,000 or 1:100,000, were given five minutes before (6 cases) or fifteen minutes after (10 cases) the test injection of horse serum. In some cases the adrenalin injections were repeated at two or three hour intervals. The mortality was no less, however, than in the experiments on suprarenalectomized rats reported above. The adrenalin seemed to exert a protective influence in only two cases. In both, two injections of 1 cc. of adrenalin, 1:100,000, were given, the first injection fifteen minutes

after the test injection of horse serum and the second two hours later. Both of these rats had no symptoms of anaphylactic shock. Eight days later a second test dose of horse serum was given to each rat. Following this one had slight symptoms and one died from anaphylactic shock 153 minutes after injection.

As it was pointed out before (Wyman, 1928), the intraperitoneal injection of adrenalin solution is a very imperfect imitation of the secretion of adrenin from the intact medulla. It is probable that further perfection of technique in a larger series of animals would give evidence that some measure of protection can be obtained in suprarenalectomized rats by the use of adrenalin. Hajós (1926) reported that sensitiveness in experimental anaphylaxis is decreased by adrenalin.

Symptoms and autopsy findings. The symptoms of anaphylactic shock in the rat, with respect to time of onset, duration and character, and the pathological findings at autopsy have been adequately described by Parker and Parker (1924). It will suffice to say that the findings in the experiments reported above were similar.

Discussion. From the results reported above it may be concluded that the suprarenalectomized rat is much more susceptible to anaphylactic shock than the normal animal. This increased susceptibility is not correlated with the degree of cortical insufficiency present after suprarenalectomy. Suprarenalectomized rats, in good condition and having as much or more healthy appearing cortical tissue in the form of autoplastic transplants or gross accessories than normal rats, are as susceptible to anaphylactic shock as those having no demonstrable cortical tissue and having various degrees of insufficiency. Since the increased susceptibility appears to be consequent to the loss of the suprarenal glands it follows that the increased susceptibility must be due to lack of medullary tissue. This is in harmony with many known facts concerning anaphylaxis and leads to the hypothesis that the suprarenal medulla is an important emergency factor in combating anaphylactic shock in normal animals.

The results reported in this paper together with the fact that the increased susceptibility to histamine of suprarenalectomized rats is largely due to the lack of medullary tissue (Wyman, 1928) are in harmony with the theory mentioned in the introduction, namely, that a factor in the anaphylactic reaction is the liberation of histamine or histamine-like substances from sensitized cells. Parker and Parker believe that in the rat the symptoms and pathology of anaphylactic shock are almost entirely due to the increased specific capillary permeability. They point out the remarkable parallelism between these effects and those due to histamine poisoning. The great natural resistance of the rat to histamine poisoning (Voegtlin and Dyer, 1925) and the relative resistance of normal rats to anaphylactic shock should also be taken into consideration.

SUMMARY

1. Of a group of twenty-five doubly suprarenalectomized rats sensitized with horse serum thirty-six per cent died from anaphylactic shock following the test dose, twenty per cent had severe symptoms, twelve per cent had moderate symptoms, and thirty-two per cent had slight symptoms. No correlation was found between the presence or absence of gross accessory cortical tissue or the time of survival after operation and the susceptibility to anaphylactic shock.

2. A group of twenty-nine normal rats sensitized with horse serum had, following the test dose, no symptoms or only slight or moderate symptoms. Of fifteen of these rats which were suprarenalectomized after the test injection twelve died of anaphylactic shock following a second test dose injected after operation.

3. Of fifteen suprarenalectomized rats having autoplastic cortical transplants, and in some cases gross accessories as well, forty per cent died from anaphylactic shock following sensitization and the injection of a test dose of horse serum.

4. An attempt to reduce the susceptibility of suprarenalectomized rats to anaphylactic shock by means of injections of adrenalin chloride solution gave suggestive though incomplete evidence of possible protection from this source.

5. It is concluded that suprarenalectomized rats are more susceptible than normal rats to anaphylactic shock, and that this increased susceptibility is not related to cortical insufficiency, but that it is consequent to the lack of medullary suprarenal tissue.

The writer is indebted to Miss Caroline tum Suden for technical assistance, and to Dr. Benjamin White, Director of the Massachusetts State Antitox'n Laboratory, for supplying the horse serum used in this work.

BIBLIOGRAPHY

Flashman, D. H. 1925. Journ. Infect. Dis., xxxviii, 461.
Hajós, C. 1926. Endocrinol., x, 560.
Lewis, T. 1927. The blood vessels of the human skin and their responses. London.
Parker, J. T. and F. Parker. 1924. Journ. Med. Res., xliv, 263.
Voegtlin, C. and H. A. Dyer. 1925. Journ. Pharm. Exper. Therap., xxiv, 101.
Wyman, L. C. 1928. This Journal, lxxxvii, 29.
Wyman, L. C. and B. S. Walker. 1929. This Journal, in press.

STUDIES ON SUPRARENAL INSUFFICIENCY

VII. Note on Temperature Regulation in Suprarenalectomized Rats

LELAND C. WYMAN and CAROLINE TUM SUDEN

From the Physiological Laboratory of Boston University School of Medicine and the Evans Memorial, No. 203

Received for publication April 29, 1929

It has been observed frequently that suprarenalectomized animals are more susceptible to cold than normal animals. A considerable body of evidence exists which indicates that one of the emergency functions of the suprarenal medulla is to put out extra adrenin in response to cold, thereby hastening combustion and assisting the sympathetic system to protect the organism against cooling by means of physical heat regulation (peripheral vasoconstriction, etc.). This evidence is summarized in the papers by Cannon, Querido, Britton and Bright (1927) and Britton (1928), and in the book by Cramer (1928). Belding and Wyman (1926) noted that suprarenalectomized rats are often less resistant to cold than normal rats. They reported an average reduction of rectal temperature of 0.7°F. in control rats and of 5.9°F. in suprarenalectomized rats after two hours in a cold room at 3°C. This paper reports some observations of a similar nature made during a series of studies on suprarenal insufficiency in the rat.

Methods. The details concerning care of the animals and general experimental and operative methods may be found in previous papers from this laboratory (see Wyman and Walker, 1929a). Three series of adult rats (5 to 12 months old) were used, ten normal rats, ten doubly suprarenalectomized rats, and ten suprarenalectomized rats having autoplastic cortical transplants in the abdominal muscles. Rectal temperatures were taken with a certified clinical thermometer, and care was taken to eliminate as far as possible errors due to variations in the depth of insertion of the thermometer, the presence of fecal masses, and struggling. All rats were gentled and made accustomed to handling and to the insertion of the thermometer by daily performance of these procedures for a considerable period before the experiments were attempted. Approximately equal numbers of both sexes were used and no consistent sex differences were noted, except that female rats tended to have a slight rise in temperature more often than male rats upon exposure to cold.

In the case of each rat two readings of the rectal temperature were taken

one hour apart with the animal at room temperature (69° to 74°F.). The rat was then placed in a cold room at a moderately low temperature (40° to 50°F.) for two hours and readings were taken at the end of the first and the second hour. The animal was then restored to room temperature and and another reading was taken after one hour. Complete autopsies were performed on all rats.

Normal rats. In ten normal, healthy rats (five males and five females) the rectal readings at room temperature varied from 97.9°F. to 100°F., averaging 99.1°F. After two hours in the cold room seven had a fall (0.3° to 1.4°F.), one had no change, and two had a rise (0.1° and 1.7°F.) in rectal temperature. The average change in rectal temperature in the cold room was a fall of 0.5°F. No particular outward reaction to the cold was shown by these rats. After one hour at room temperature following the exposure to cold all had recovered their original temperatures.

Suprarenalectomized rats. A similar experiment was done on ten doubly suprarenalectomized rats from 29 to 39 days after operation. Five of these were found later at autopsy to have one or two gross accessory masses of healthy appearing cortical tissue from 1½ to 8 mm. in diameter. No signs of accessory chromaffin tissue could be found. Abundant abdominal fat was present. The rectal temperatures of these five at room temperature averaged 98.4°F. After two hours in the cold room three had a fall (0.3° to 2.2°F.) and two had a rise (0.1° and 0.7°F.) in rectal temperature. The average change in the cold room was a fall of 0.62°F. No external reaction to the cold was noted. After one hour at room temperature all had recovered their original temperatures with the exception of the one which had had a fall of 2.2°F.

The rat last mentioned recovered its original temperature after two hours. One other rat was found at autopsy to have a small gross accessory cortical mass, and after two hours in the cold room it had a fall of 4.4°F. During the exposure to cold it shivered, felt cold to touch, and showed signs of discomfort, and following this after one hour at room temperature it had not recovered its original temperature. These two rats were killed and the accessory cortical masses were found 119 days after the experiment described above. It is possible that at the time of the experiment the accessory masses had not become established as functioning cortical tissue.

The other four rats in this series must be described individually. One showed symptoms of suprarenal insufficiency when placed in the cold room (diarrhea, asthenia, etc.). After the first hour the rectal temperature had fallen 6.8°F. and after the second hour it had fallen 7.2°F. During the exposure to cold the rat was feeble and felt cold to touch. After one hour at room temperature following exposure to cold the rectal temperature had fallen another degree (89°F.) and later in the day the rat died and

typical findings of suprarenal insufficiency were seen at autopsy. Another rat had a low reading at room temperature (95°F.) and upon exposure to cold showed marked reactions such as shivering, erection of hair, weakness and sluggishness. After two hours in the cold room the rectal temperature had fallen 4.2°F., and following this after two hours at room temperature the rectal reading had risen only 1.4°F. The next morning the rectal temperature was back to 95.6°F. Five days later this rat died with typical symptoms and autopsy findings of suprarenal insufficiency. Another rat showed moderate reactions to the cold and after two hours in the cold room had a fall in rectal temperature of 2.2°F. This rat recovered its original temperature after one hour at room temperature. Fifty-seven days later it died with typical symptoms and autopsy findings of suprarenal insufficiency. The remaining rat showed only slight reactions to the cold, had a fall of 0.9°F. after two hours in the cold room, and recovered its original temperature after one hour at room temperature. This rat was killed for autopsy 118 days later and no gross accessory suprarenal tissue could be found. An inspection of these results shows that the fall of body temperature upon exposure to moderate cold and the lack of ability to rapidly recover normal temperature is correlated with the degree of cortical insufficiency which is present.

Rats with autoplastic cortical transplants. In ten suprarenalectomized rats having autoplastic cortical transplants the rectal readings at room temperature varied from 96.2°F. to 98.7°F., averaging 97.7°F. After two hours in the cold room four had a fall (0.2° to 0.4°F.), one had no change, and five had a rise (0.3° to 1.4°F.) in rectal temperature. The average change was a rise of 0.32°F. No marked external reactions to the cold were seen in these rats. After one hour at room temperature following the exposure to cold all had recovered their original temperatures. This experiment was done from 94 to 119 days after operation. All these rats were fat and healthy and later at autopsy from one to three well vascularized masses of cortical tissue, from 1.5 to 5 mm. in diameter, were found at the sites of transplantation. No signs of chromaffin tissue could be found.

Discussion. The results reported above indicate that in suprarenalectomized rats inability to maintain a normal body temperature upon exposure to moderate cold is correlated with cortical insufficiency. The suprarenal cortex, as well as the medulla, appears to be concerned with heat regulation. The relationship is probably indirect since it is known that metabolic disturbances are present in cortical insufficiency. There is considerable evidence that in suprarenal insufficiency there is a concentration of the blood, probably through the loss of plasma because of increased permeability of blood vessel walls. The importance of water-shifting in the body and of changes in the fluid concentration of the blood

in physical heat regulation has long been pointed out (see Barbour, 1921). With a permanently concentrated blood a disturbance in physical heat regulation is to be expected.

The supposed rôle of the suprarenal gland in heat regulation is in harmony with the suggestion (Wyman and Walker, 1929b) that the suprarenal cortex is concerned in the steady maintenance of certain bodily conditions, while the medulla brings about rapid adjustments in the same direction under emergency conditions. Although lack of medullary tissue did not appear to interfere with the maintenance of body temperature upon exposure to moderate degrees of cold in the experiments with animals having gross cortical accessories or cortical transplants, further work is indicated to determine the critical temperature at which lack of the medulla in rats would result in difficulty in temperature regulation.

SUMMARY

1. After two hours in a moderately cold room normal rats, suprarenalectomized rats with autoplastic cortical transplants, and suprarenalectomized rats having gross accessory cortical masses (with two exceptions) have similar slight variations in the rectal temperature. All these have no marked external reactions to the cold and recover their original normal temperatures after one hour at room temperature following exposure to cold.

2. Rats having cortical insufficiency show a fall in the rectal temperature after two hours in the cold room, show more or less marked external reactions to the cold, and do not recover their original temperatures in a warm room as soon as those having cortical tissue. The fall of rectal temperature upon exposure to cold and the inability to rapidly recover normal temperature is correlated with the degree of cortical insufficiency which is present.

3. It is concluded that the suprarenal cortex, as well as the medulla, is indirectly concerned with heat regulation.

BIBLIOGRAPHY

Barbour, H. G. 1921. Physiol. Reviews, i, 295.

Belding, D. L. and L. C. Wyman. 1926. This Journal, lxxviii, 50.

Britton, S. W. 1928. This Journal, lxxxiv, 119.

Cannon, W. B., A. Querido, S. W. Britton and E. M. Bright. 1927. This Journal, lxxix, 466.

Cramer, W. 1928. Fever, heat regulation, climate, and the thyroid-adrenal apparatus. London.

Wyman, L. C. and B. S. Walker, 1929a. This Journal, in press.

Wyman, L. C. and B. S. Walker, 1929b. This Journal, in press.

PHYSIOLOGICAL VARIATIONS IN THE CARDIAC OUTPUT OF MAN

III. The Effect of the Ingestion of Food on the Cardiac Output, Pulse Rate, Blood Pressure, and Oxygen Consumption of Man

ARTHUR GROLLMAN

From the Physiological Laboratory, School of Medicine, The Johns Hopkins University

Received for publication April 18, 1929

The effect of the ingestion of food on the cardiac output has been previously considered by Collett and Liljestrand (1924), Kisch and Schwarz (1925), and Jarisch and Liljestrand (1927). Unfortunately, the methods employed by these authors involved rather large errors. Hence, reliable conclusions as to the exact nature of the circulatory adjustments following meals can not be made from the data available at present. The problem is of vast importance physiologically because of the light it throws on the mechanism of the circulatory changes following the ingestion of food. Moreover, it is necessary to know how food affects the blood flow, in order to make possible the evaluation of data obtained under other than true basal conditions.

Methods. The method employed was that described in previous publications (Marshall and Grollman, 1928; Grollman, 1928, 1929a, b). The experiments were carried out in the afternoon 5 or 6 hours after a light breakfast and after the subject had rested completely relaxed in a steamer chair for 45 minutes. Under these conditions the cardiac output was found to have the same value as when taken under basal conditions, i.e., in the morning and 15 hours after the last meal. After determining the basal values, the food was brought to the subject without necessitating his movement from the steamer chair. The determinations were then continued throughout the afternoon. In order to avoid the effect of fluids on the cardiac output, the fluid intake was limited to less than 250 cc. which amount, if taken alone, has been found (Grollman, 1929b) to be without effect on the cardiac output.

Results. A series of 12 determinations on 6 individuals gave results which were perfectly uniform. In each case there was a rapid rise of 0.5 to 2.0 liters in the cardiac output, the magnitude of this rise being dependent upon the amount of food ingested. The cardiac output then remained elevated for an hour or more before it began to decrease. It reached its maximum value before the oxygen consumption had reached

its maximum value. The arterio-venous oxygen difference dropped immediately after the ingestion of the food and then gradually rose throughout the remainder of the experiment. It reached its fasting level before the oxygen consumption had returned to its basal value. Since all of the results obtained were perfectly uniform, only two typical protocols need be cited as examples of the results obtained. Protocol 1 gives the results obtained after eating as heavy a meal as the subject could comfortably consume. Protocol 2 shows the results obtained after a comparatively light luncheon. In figure 1 the changes in the various physiological functions studied, as found in a typical experiment, are graphically illustrated.

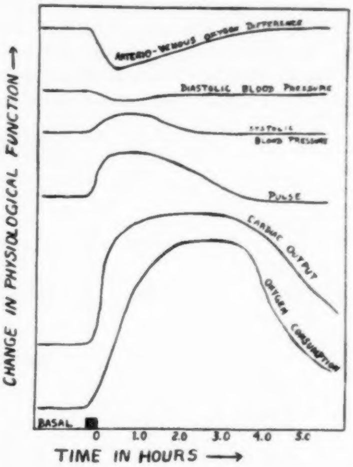


Fig. 1. Graphic representation of the changes in various physiological functions as a result of the ingestion of a heavy meal. The shaded area on the horizontal axis represents the time during which the food was being ingested. The horizontal portions of the curves, to the left of the shaded area, give the basal values. The abscissae represent the time, in hours, after the ingestion of the food; the ordinates represent the observed changes in the various physiological functions studied.

Discussion. Recent reviews of the extensive literature on the effect of food on the pulse and blood pressure (Rihl, 1926; Fleisch, 1927; Jarisch and Liljestrand, 1927) render it unnecessary to consider in detail the changes in these functions observed during the present investigation. The pulse always rose immediately after the ingestion of food but usually fell to its original value or even lower (as in the case of protocol 2) within a short time. Since the cardiac output, on the other hand, remained elevated for some hours, the stroke volume is seen to be a very variable function during the course of the experiment, being usually greatly increased by the intake of food.

The systolic pressure always rose rather slightly in accord with the findings of Weysse and Lutz (1915) while the diastolic pressure remained unchanged or fell as observed by Janeway (1904). The pulse pressure thus rose slightly as a result of the intake of food. These results are easily explicable on the basis of the observed increased cardiac outputs and a widespread vaso-dilatation in the splanchnic area after the ingestion of food.

The cardiac output as seen in protocols 1 and 2 is greatly affected by

the ingestion of food. It rises immediately and reaches a maximum value at about a half-hour after the meal. This maximum is then sustained for an hour (in the case of a light luncheon) to three hours (in the case of a heavy meal). The maximum increase observed in the cardiac output was 0.5 to 0.9 liter after a light meal and 1.0 to 2.0 liters after a heavy

Protocol 1. The effect of a heavy meal on the cardiac output, pulse, blood pressure, and oxygen consumption. Subject: L. M., ♂, age 25 years. Height, 168 cm. Wt. 57 kgm. The subject had eaten a light breakfast at 7:30 a.m. and had indulged in moderate activity during the forenoon. The subject rested in a steamer chair from 11:10 a.m. From 12:30 to 12:53 p.m. the subject ate a very heavy meal consisting of bread, meat, sweet chocolate, and 150 cc. of milk.

TIME	PULSE	BLOOD PRESSURE	OXYGEN CONSUMPTION	ARTERIO-VENOUS OXYGEN DIFFERENCE	CARDIAC OUTPUT
			cc. per minute	*cc. per liter*	*liters per minute*
12:14			206		
12:22	74	95/62			
12:27				60.0	3.43
12:55	80	102/60			
1:00			239		
1:07				50.6	4.72
1:20	78	98/56			
1:30				53.6	4.66
1:36			250		
1:40	80				
2:05	75	95/58			
2:10				55.0	4.64
2:16			255		
2:48			269		
2:53	75	100/56			
2:56				58.8	4.57
3:39			270		
3:45	73				
3:46				57.8	4.67
4:20			253		
4:30	69	94/58			
4:35				60.1	4.06
5:00	70	95/58	235		
5:05				60.0	3.92

meal. Five hours after the ingestion of breakfast or a light luncheon, the cardiac output had returned to its fasting level.

We may visualize the circulatory changes following the ingestion of food as consisting of a dilatation of the splanchnic vessels with a concomitant increase in the output of the heart which maintains the blood pressure constant or but slightly changed.

The increases in cardiac output after meals, as observed in this study,

are much less than that found by Kisch and Schwarz (1925). These increases are, however, much greater than the increases found after the ingestion of large quantities of water (Grollman, 1929b), the reverse of this being reported by Jarisch and Liljestrand (1927). Collett and Liljestrand (1924) found that the value of the cardiac output depended upon

Protocol 2. The effect of a light lunch on the cardiac output, pulse, blood pressure, and oxygen consumption. Subject: A. G., ♂. Age 27 years. Height, 164 cm., wt. 63 kgm. The subject ate a light breakfast at 7:30 a.m.; indulged in moderate activity during the forenoon; and rested from 12:40 p.m. From 1:43 to 1:47 the subject ate a sandwich (bread and meat) and a cup of coffee (containing sugar and cream).

TIME	PULSE	BLOOD PRESSURE	OXYGEN CONSUMPTION	ARTERIO-VENOUS OXYGEN DIFFERENCE	CARDIAC OUTPUT
			cc. per minute	*cc. per liter*	*liters per minute*
1:25	80	119/86			
1:32			237		
1:38	80	117/89			
1:40				64.8	3.66
1:50	88	117/82			
1:53				59.4	4.26
2:00			253		
2:06	84	124/87			
2:13				57.6	4.39
2:23	80	118/78			
2:28			253		
2:40	80	122/83			
2:45				62.7	4.15
2:55	82	113/80			
3:00			266		
3:15	82	108/78			
3:20				65.0	4.11
3:40			268		
3:55	74	108/76			
4:00				63.9	4.07
4:10	74	118/81			
4:18			252		
4:30	74	114/86			
4:40				62.0	3.89
4:47			241		

the level of the fasting minute volume for the day. Since no such diurnal variation[1] has been encountered in our investigation, the present results do not confirm this view. In the case of one of the two subjects whom Collett and Liljestrand studied, practically the same increase in cardiac

[1] This question will be considered in detail in a later communication dealing with diurnal variations of the cardiac output.

output followed a light as a heavy meal. Such a result we attribute to the particularly large error in the measurements on their subject, a fact also previously noted in the study of the effect of posture on the cardiac output (Grollman, 1928).

SUMMARY

The changes in the pulse rate, blood pressure, oxygen consumption and cardiac output of 6 subjects were studied after the ingestion of light and heavy meals.

After the ingestion of food the pulse rises, but falls within a short time to the resting value.

The systolic blood pressure usually rises somewhat while the diastolic pressure remains constant or falls slightly. The pulse pressure is, therefore, but slightly increased.

The cardiac output rises immediately after the ingestion of food, reaches a maximum of 0.5 to 2.0 liters over the fasting level, and remains at this high and practically constant level for 1 to 3 hours. Four or five hours after the ingestion of a light meal the cardiac output has returned to its fasting level.

BIBLIOGRAPHY

Collett, M. E. and G. Liljestrand. 1924. Skand. Arch. f. Physiol., xlv, 25.

Fleisch, A. 1927. In Handbuch der normalen u. pathologischen Physiologie. Berlin, vii, 1278.

Grollman, A. 1928. This Journal, lxxxvi, 285.

1929a. Ibid., lxxxviii, 432.

1929b. Ibid., lxxxix, 157.

Janeway, T. C. 1904. The clinical study of blood pressure. New York and London, p. 117.

Jarisch, A. and G. Liljestrand. 1927. Skand. Arch. f. Physiol., li, 235.

Kisch, F. and H. Schwarz. 1925. Ergebn. der inn. Med. u. Kinderheilk., xxvii, 198.

Marshall, E. K. and A. Grollman. 1928. This Journal, lxxxvi, 117.

Rihl, J. 1926. In Handbuch der normalen u. pathologischen Physiologie. Berlin, vii, 505.

Weysse, A. W. and B. R. Lutz. 1925. This Journal, xxxvii, 303.

A COMPARISON OF ANTERIOR HYPOPHYSEAL IMPLANTS FROM NORMAL AND GONADECTOMIZED ANIMALS WITH REFERENCE TO THEIR CAPACITY TO STIMULATE THE IMMATURE OVARY[1]

HERBERT M. EVANS AND MIRIAM E. SIMPSON

From the Department of Anatomy, University of California

Received for publication April 22, 1929

About three years ago Zondek and Aschheim and P. E. Smith independently discovered the existence of a hormone in the anterior hypophysis of mammals which markedly stimulated the sexual apparatus of immature animals, bringing young females, for instance, into complete sexual ripeness[2] within four days. The method consists in the physical introduction or "implantation" of bits of anterior hypophyseal tissue by any parenteral route, preferably intramuscularly. The method would appear capable of giving us information regarding differences in the hormone content of these little glands under various physiological and pathological conditions. The manifest relation of the hypophysis to the genitals suggests at once experiments to disclose alteration in this hormone as the result of gonadectomy, a procedure already known to mysteriously enlarge the size of the hypophysis.

We selected a large group of healthy adult male and female rats all seven to eight months of age. Half of the females were gonadectomized and half left normal, care being taken that littermate sisters, or animals of the same weight and age were distributed equally in the two groups. The males were divided into three groups: One-third were gonadectomized, one-third were rendered cryptorchid by abdominal anchorage of the testes, and a third were left normal. Exactly two months later the anterior hypophyses from the two types of female and three types of male donors were implanted intramuscularly into young female littermate rats twenty-four days of age. In each case two adult glands were given daily for two days, to each young recipient. The young host animals were watched closely for signs of maturity and killed at the end of the fourth day; their ovaries and uteri were weighed and examined histologically.

[1] Aided by grants from the Committee for Research in Problems of Sex of the National Research Council and from the Board of Research of the University of California.

[2] Smith and Engle first demonstrated the actual discharge of large numbers of eggs (Amer. Journ. Anat., Nov. 15, 1927, xl, 159).

TABLE 1

Showing increase in sex hormone content of the anterior hypophysis of gonadectomized rats of both sexes two months after the operation, each recipient receiving the anterior lobes from four glands

	TYPE OF DONOR FROM WHICH HYPOPHYSEAL IMPLANTS WERE TAKEN					
	None (Controls)	Normal females	Gonadectomized females	Normal males	Gonadectomized males	Cryptorchid males
Average weight in milligrams of the four hypophyses used for implants	None given	47.6	49.3	34.4	48.8	43.6
Percentage of recipients showing oestrous vaginal smear within 4 days	0%	16%	100%	100%	100%	100%
Individual and average weights in milligrams of both ovaries of young host at end of fourth day	16 14 15 16 17 19 21 18 Average 17	25 17 16 24 14 12 28 21 17 13 21 26 Average 19.5	137 97 110 148 87 102 Average 113.5	90 108 127 40 42 46 94 57 28 69 83 50 Average 69.5	166 207 152 165 226 140 Average 176	121 147 95 115 130 167 Average 129

The subjoined table (table 1) depicts the results of these experiments. The anterior hypophyses of the gonadectomized females at the level indicated provoke ovaries weighing 113.5 mgm. in the little hosts, approximately six times the weight of the ovaries in their sisters which received the same implantation treatment with four normal adult female hypophyses. The hypophyses of castrated males were also more effective

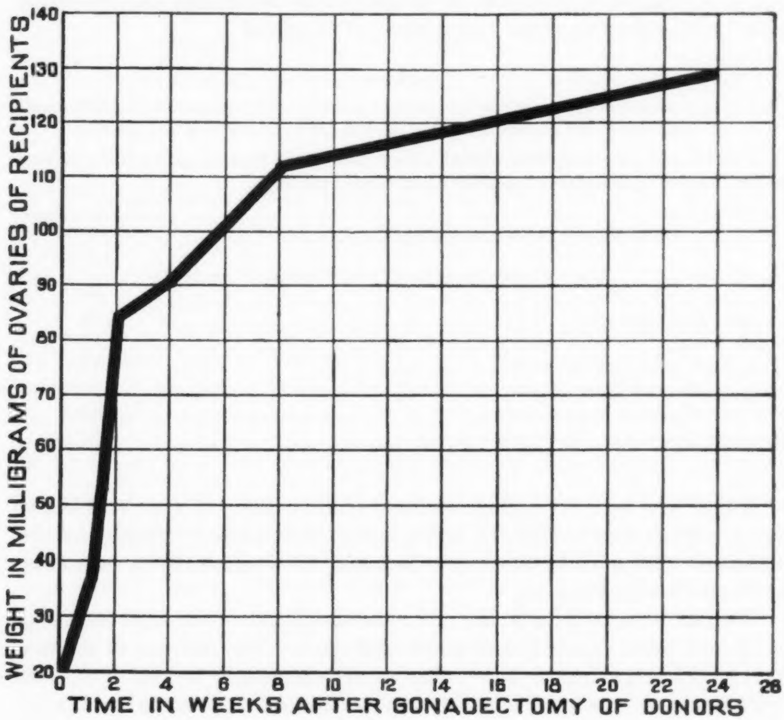


Fig. 1. Graph showing rate of increase in hormone content of the hypophyses of spayed adult females as determined by the weight of the ovaries produced by the intramuscular implantation of these hypophyses into twenty-four day old females.

than those from normal males, the young ovaries attaining their greatest weight in these cases, an average of 176 mgm. or some two and one-half times the weight of the ovaries of their sisters implanted with normal male hypophyses. The cryptorchid male hypophyses gave results intermediate between those for normal and castrated males. Since the cryptorchid animals preserved their sex interest and copulatory ability but gradually lost their germ cells (chiefly within the first month), the results indicate

that it is to the germ cell component of the testis rather than to the interstitial tissue that the anterior hypophysis is directly related. The results as a whole would appear to indicate clearly either increased production or defective utilization and hence storage of the sex hormone of the anterior hypophysis after castration. The histological picture in the heavier hypophyses of gonadectomized animals could possibly be interpreted as due to storage of the hormone on the part of the basophiles (the castration cells[3]) rather than to a true hypertrophy of the gland.

TABLE 2

Rate of accumulation of the sex hormone in the hypophysis of gonadectomized female rats as indicated by the average weight of the ovaries in four young test females

Two hypophyses daily were implanted on each of the first two days of the experiment, the young recipients being killed on the fourth day

TYPE OF DONOR OF ANTERIOR HYPOPHYSEAL IMPLANTS	AVERAGE WEIGHT OF BOTH OVARIES OF YOUNG RECIPIENT
	mgm.
Normal adult females	16
Adult females one week after gonadectomy	38
Two weeks after gonadectomy	84
One month after gonadectomy	92
Two months after gonadectomy	113.5
Six to eight months after gonadectomy	130.8

If this view be correct, then the functional integrity of male and female germ cells is essential for the utilization of the anterior hypophyseal sex hormone. In gonadectomy the hormone thus gradually accumulates until true atrophy sets in.

We have secured data on the rate of accumulation of the hormone as the subjoined table (table 2) and graph will show. The increase in the first two weeks is much more rapid than in the succeeding two, after which a very slow pace sets in which is apparently maintained for some months.

[3] Gertrude van Wagenen: Anat. Record, 1925, xxix, 398.

A SEX DIFFERENCE IN THE HORMONE CONTENT OF THE ANTERIOR HYPOPHYSIS OF THE RAT[1]

HERBERT M. EVANS AND MIRIAM E. SIMPSON

From the Department of Anatomy, University of California

Received for publication April 22, 1929

It has been known for many years that the hypophysis in the female is absolutely, and of course relatively, heavier than in the male. A special burden falls on the gland in the female, a fact depicted by its increased size and histologic change during pregnancy and by the recent demonstration by Zondek and Aschheim[2] of the immensely increased outpouring of one of its hormones at this time. Investigators have also pointed out the increase in weight of the gland in multipara. Any notion, however, that the increased weight of the female gland is directly attributable to the reproductive process must be abandoned when we observe practically the same difference between males and virgin females. In the careful studies conducted by Donaldson and his school[3] on the rat these facts come out clearly. In our own hybrid Norway-albino rat strain, we have found, for instance, that 8.8 mgm. was the average weight of the hypophysis from 92 males whose average body weight was 325 grams and that 11.6 mgm. was the average weight of the hypophysis of 84 females whose body weight averaged 234 grams. The very recent work of Smith and Engle[4] showing fluctuations in the hormone content of the gland correlated with the phase of the oestrous cycle is strongly suggestive of a frequently recurring periodic function throughout life independent of pregnancy, so that this might be looked upon as provocative of the greater gland in the female. Finally, the very clear effect of the anterior hypophyseal sex hormone in provoking precocious maturity in young females might induce us to believe

[1] Aided by grants from the Committee for Research in Problems of Sex of the National Research Council and from the Board of Research of the University of California.

[2] Zondek and Aschheim: Klin. Wochenschr. 1927, no. 28.

[3] Donaldson, H. H.: The Rat. Mem. of the Wistar Institute of Anatomy and Biology, no. 6, Phila., 1924, xiv, 469, 8°.

Hatai: 1913. Amer. Journ. Anat., xv, 87.

Hatai: 1913. Journ. Exper. Zoöl. xv, 297.

Hatai: 1914. Anat. Record, viii, 511.

[4] P. E. Smith and E. T. Engle: Anat. Record, xlii, no. 1, March 1929, 38.

that the ovary-hypophysis relationship is more pronounced than is that of the testis and hypophysis.

All of the foregoing facts lend a peculiar interest to efforts to compare the hormone strength of the male and female anterior hypophysis as tested by the efficacy of implants in inducing precocious maturity of young females. It can be taken for granted that animals of the same age or weight, preferably, indeed, littermates, should be used both for donors and for recipients in a well conducted experiment. The data presented below indicate very clearly a greater potency in the male gland. These facts should lead us, we believe, to recognize the hypophysis-testis relationship as equally important and in some respects more emphatically expressed than are these important relations in the female sex.

Determination of hormone unit as the minimal effective dose. We may designate the hormone unit in experiments such as ours as that minimal quantity administered to immature rats which will in one-half of all cases within a total period of four days lead to the breakdown of the vaginal closing membrane, the appearance of the pro-oestrous and oestrous vaginal smear and appreciable increase in the weight or presence of corpora lutea in the infantile ovary. By the use of large numbers of animals we have determined the amount of dosage meeting these criteria when the anterior hypophyseal tissue is taken from adult female donors weighing from 200 to 300 grams. The pars anterior from six of these glands, which weigh in toto 67 mgm., two given daily for three days represent such a minimal "implant" dose. In all cases the recipient animals are females twenty-four or twenty-five days of age at the initiation of the experiment and aged twenty-eight to twenty-nine days at its close. Our strain is a Norway-albino mixture, the young females averaging 46 to 50 grams in weight on the first day of the test. Implants are made deep in the limb musculature. Sacrifice of the young recipients at the ninety-sixth hour of the experiment and observation and weight of their ovaries together with serial sectioning of the latter completes the evidence for the degree of stimulation imparted to the young gonads.

The subjoined tables (see tables 1 and 2) give the average weight of the infantile ovaries in animals sacrificed at the completion of the fourth day of experimentation after various implantations of adult male and female anterior hypophyseal lobes.

These tables demonstrate clearly that three male hypophyses produce changes in the immature ovary as great as those produced with five or six female hypophyseal implants.

The increased potency of the male gland was so marked that we were led to investigate the effect of glands from immature males. For this purpose pituitaries of young males three or four days after weaning were implanted into the limb musculature of their sisters. The results are

shown in table 3. They demonstrate that judging by the weight of the young ovaries produced, the immature male pituitary is more than twice as potent as that of the adult female.

Attention has already been drawn by Engle to the increased potency of the hypophysis from gonadectomized animals, and confirming him, our

TABLE 1

Female hypophyseal implants

NUMBER OF RECIPIENTS	TOTAL NUMBER OF ADULT ANTERIOR HYPOPHYSES IMPLANTED IN EACH RECIPIENT	PER CENT OF RECIPIENTS SHOWING OESTROUS VAGINAL SMEAR	AVERAGE WEIGHT OF THE TWO OVARIES AT THE END OF THE FOURTH DAY
			mgm.
29	None (controls)		16
6	3	16	16
34	4	50	19.7
4	5	75	23
6	6	100	29

TABLE 2

Male hypophyseal implants

NUMBER OF RECIPIENTS	TOTAL NUMBER OF ADULT ANTERIOR HYPOPHYSES IMPLANTED IN EACH RECIPIENT	PER CENT OF RECIPIENTS SHOWING OESTROUS VAGINAL SMEAR	AVERAGE WEIGHT OF THE TWO OVARIES AT THE END OF THE FOURTH DAY
			mgm.
4	3	100	24.5
12	4	100	70
4	5	100	79

TABLE 3

Twenty-four-day-old male hypophyseal implants

NUMBER OF CASES	TOTAL NUMBER OF ANTERIOR GLANDS IMPLANTED	WEIGHT OF DONOR	PER CENT OF RECIPIENTS SHOWING OESTROUS VAGINAL SMEAR	AVERAGE WEIGHT OF THE TWO OVARIES AT END OF FOURTH DAY
		grams		*mgm.*
3	2	50–60	0	16
3	4	50–60	66	41
3	6	50–60	100	60

preceding paper in this journal will have established, we trust, the invariable rise in potency of the implants from gonadectomized as contrasted with normal donors of both sexes. While glands from gonadectomized males are more potent than those of gonadectomized females (about one and one-half times) their superiority is not proportionately as great as it is

in the cases from normal animals of both sexes, where we may regard the male gland as about three and a half times more effective than the female. Under the condition of that experiment the hypophyses of gonadectomized females had increased almost six times in potency whereas those of gonadectomized males had been rendered only approximately two and one-half times more effective.

It seems impossible to regard the wide difference in efficacy of male and female normal glands as indicating anything but increased hormone storage and superior rate of hormone production in the normal male. But if this be the case, our experiments show clearly that gonadectomy abruptly halts the superior male rate. For if there were a temporary survival of the greater male rate, the glands from gonadectomized male donors would be even more increased in potency than is actually the case. The increased young ovarian tissue produced by taking glands from gonadectomized as contrasted with normal male donors is about the same as is the case in experiments with the two types of female donors. If we assume that the gland of the gonadectomized male does not lose the hormone with which it started, then it appears that both male and female glands from gonadectomized animals store additional hormone at practically the same rate.

THE EFFECT OF PREGNANCY ON THE ANTERIOR HYPOPHYSIS OF THE RAT AND COW AS JUDGED BY THE CAPACITY OF IMPLANTS TO PRODUCE PRECOCIOUS MATURITY[1]

HERBERT M. EVANS AND MIRIAM E. SIMPSON

Department of Anatomy, University of California

Received for publication April 22, 1929

The singular enlargement of the hypophysis in pregnancy (due to the presence of the so-called "pregnancy cells") and the existence in the urine of pregnant women of a hormone presumably from the anterior hypophysis, both suggest that implantation of anterior hypophyseal tissue from pregnant donors would disclose increased amounts of the sex hormone in the glandule at this time. We determined the minimal amount of anterior hypophyseal tissue fron non-pregnant cows which, given in three daily doses by the implant method, would provoke the oestrous vaginal smear and development of the ovaries in twenty-four day old female rats sacrificed on the fourth day. This amounted to approximately 35 milligrams daily. The ovaries in the young rat recipients always contained several corpora lutea and averaged about 30 mgm. in weight (in five cases actually 32, 32, 27, 33, and 19 mgm.). The identical technique with anterior hypophyseal tissue from pregnant cows gave essentially similar findings, the ovaries being somewhat, but not much, heavier—averaging 37 mgm. (actually 38, 38, 28, 48, and 34 mgm.).

We next sought to determine the effect of pregnancy on the maturity hormone content of the rat hypophysis as judged by its power of awakening a precocious maturity in young females. We had already determined that the anterior lobes from four normal female rats given as implants (two daily for two days) constituted slightly less than the minimal effective dose and so selected this level of dosage and one a little below it (three glandules, given as one daily implant for three days) as best calculated to show the slightest increase in the potency of the hypophyses from pregnant animals. The subjoined table (table 1) shows no significant difference in the results from three as contrasted with four glandules but at both levels it again demonstrates an indubitable though very slight increase of hormone in the glandules from pregnant donors. The hypophyses from multiparous

[1] Aided by grants from the Committee for Research in Problems of Sex of the National Research Council and from the Board of Research of the University of California.

TABLE 1

Effect of pregnancy on the sex hormone content of the rat's hypophysis

TYPES OF IMPLANTS (ANTERIOR LOBE ONLY)	PERCENTAGE OF RECIPIENTS SHOWING OESTROUS VAGINAL SMEAR WITHIN FOUR DAYS	WEIGHT OF BOTH OVARIES OF YOUNG RECIPIENTS	AVERAGE
	per cent	*mgm.*	*mgm.*
Three rat hypophyses (one daily for three days) *non-pregnant* donors	16	18, 18, 20, 14, 14, 14	16.5
Three rat hypophyses (one daily for three days) *Pregnant* donors (17th day of pregnancy)	66	20, 14, 22, 18, 14, 24, 24	22.6
Four rat hypophyses (two daily for two days) *non-pregnant* donors	33	18, 32, 22, 22, 14, 18	21.0
Four rat hypophyses (two daily for two days) *Pregnant* donors	66	22, 20, 26, 20, 18, 26	22.0

TABLE 2

Effect of previous pregnancies on the sex hormone content of the rat's hypophysis

TYPE OF IMPLANTS (ANTERIOR LOBE ONLY)	PERCENTAGE OF RECIPIENTS SHOWING OESTROUS VAGINAL SMEAR WITHIN FOUR DAYS	WEIGHT OF BOTH OVARIES OF YOUNG RECIPIENTS	AVERAGE
	per cent	*mgm.*	*mgm.*
Four rat hypophyses (two daily for two days) non-pregnant *nullipara*	0	16, 18, 13, 18	16.2
Four rat hypophyses (two daily for two days) non-pregnant *multipara*	75	10, 14, 16, 38	19.5

non-pregnant donors are also very slightly more potent in awakening the precocious maturity of young recipients than those from nulliparous donors, as table 2 will show.

The increase in hormone content of glands from pregnant as contrasted with non-pregnant cases and from multipara as contrasted with virgins is surprisingly little. This fact becomes more striking when we remember the far greater potency of hypophyseal implants from gonadectomized animals as compared with normal animals and the greater potency of hypophyses from males as compared with females (see the prceding papers of this series).

A COMPARISON OF THE OVARIAN CHANGES PRODUCED IN IMMATURE ANIMALS BY IMPLANTS OF HYPOPHYSEAL TISSUE AND HORMONE FROM THE URINE OF PREGNANT WOMEN[1]

HERBERT M. EVANS AND MIRIAM E. SIMPSON

From the Department of Anatomy, University of California

Received for publication April 23, 1929

In young rats implantation of fresh rat hypophysis and injection of hypophyseal hormone extracts from the urine of pregnant women cause rupture of the vaginal closing membrane, cornified cells in the vaginal smear, development of the uterus and distention with fluid within 96 hours. In so far the reaction is the same as that produced by the injection of folliculin. These methods differ from folliculin injection, however, in that the uterine and vaginal changes are secondary and the ovarian development is the primary change. The methods of administering the hypophyseal hormone referred to above are alike in that they stimulate follicular development in the infantile ovary to the point where enough folliculin is produced to cause characteristic changes in the uterus and vagina. The reaction of the immature ovary to rat hypophysis implants and to potent extracts differs, however, in several important respects.

In the first place there are the differences in the weights attained by the ovaries in a standard time. Although the minimum dose[2] of rat implant produces an ovary no heavier than the minimal dose of extract, increase in implant dosage above the minimum results in the production of ovaries varying in weight roughly in proportion to the amount of implant. If four times the minimum dose of implant[3] is given, the ovaries weigh four times more than those stimulated the same length of time by the minimal dose. On the other hand, the minimal dose of extracts can be increased 32- or

[1] Aided by grants from the Committee for Research in Problems of Sex of the National Research Council and from the Board of Research of the University of California.

[2] By minimal dose is meant the lowest dose which in 50 per cent of the cases will cause vaginal membrane rupture and will stimulate the development of follicles and the production of corpora or corpora-like bodies in four days. The presence of hemorrhagic spots has not been found to have the importance in rats ascribed to it in mice by Zondek.

[3] It is understood the implants are of a uniform nature—all from male or all from female donors and that they are from animals of approximately the same body weight.

even 50- and 160-fold and the weight of the ovaries is barely doubled or trebled (see tables 1 and 2).

The difference in size of the ovaries resulting from the two types of treatment might be considered as due to the fact that the extract doses were not distributed throughout the day but were usually given in a single dose. Under these circumstances, the ovary may be stimulated only intermittently; probably much of the hormone which is not utilized at the moment is lost through the urine. The ovary of the implanted animal may on the other hand be stimulated continuously by the small amounts of hormone diffusing from the gland. When the point was tested by contrasting the effect of a medium sized dose of extract, $\frac{3}{4}$ cc. given as three distributed doses daily of $\frac{4}{32}$ cc. each for two days and given as three single doses daily

TABLE 1

Showing increase in weight of ovaries stimulated by increasing hypophyseal implant dosages

AMOUNT OF ANTERIOR HYPOPHYSEAL TISSUE IMPLANTED	DISTRIBUTION OF IMPLANT DOSAGE	TYPE OF RATS FROM WHICH HYPOPHYSEAL IMPLANTS WERE TAKEN	NUMBER OF CASES	RECIPIENTS SHOWING OESTROUS VAGINAL SMEAR BY THE 4TH DAY	AVERAGE WEIGHT OF 2 OVARIES OF RECIPIENTS ON THE 4TH DAY
				per cent	*mgm.*
½ lobe	1 dose	Male, castrated 1 month	6	50	21
1 lobe	1 dose	Male, castrated 1 month	10	100	49
2 lobes	1 daily, 2 days	Male, castrated 1 month	10	90	68
4 lobes	2 daily, 2 days	Male, castrated 1 month	10	90	105
2 lobes	1 daily, 2 days	Males, weighing 50–60 grams	3	0	16
4 lobes	2 daily, 2 days	Males, weighing 50–60 grams	3	66	41
6 lobes	2 daily, 3 days	Males, weighing 50–60 grams	3	100	60

of $\frac{1}{4}$ cc. each, it was found that the weights of the ovaries resulting averaged 20.8 mgm. and 20 mgm. respectively. The difference in stimulation of the ovaries was then insignificant. The same slight difference was shown by comparing the different methods of administration of large doses. As can be seen in table 2, 2 cc. single doses daily for three days of AA 37 gave ovaries weighing 34 mgm. The same volume daily of a solution of the same potency (AA mixture, see table 3) given in three distributed doses daily for three days gave ovaries weighing 37 mgm. Distribution of doses to the extent of three daily dosages did not, then, materially affect the result. It was feared that a distribution of three times daily during daylight hours was not an adequate test of the point, so a searching test of the efficacy of distributed doses was made. The animals received 2 cc. daily amounts of solutions potent in $\frac{3}{16}$ cc. and $\frac{3}{32}$ cc. The extracts were given as single

daily doses and as distributed doses at $1\frac{1}{2}$ hour intervals. As can be seen in table 3, under AA mixture and AA 46, even this almost continuous dosage did not stimulate the ovaries to a much greater development than was attained by the same total amount given as single daily doses.

Amounts of hormone (see AA 53, table 3) 160 times the minimal dose,

TABLE 2

Showing the insignificant increase in the weights of ovaries after greatly increased dosage of extracts of the urine of pregnant women

DESIGNATION OF EXTRACT	DOSAGE, GIVEN INTRAMUSCULARLY OR SUBCUTANEOUSLY	TOTAL AMOUNT GIVEN	NUMBER OF CASES	RECIPIENTS SHOWING OESTROUS VAGINAL SMEAR		WEIGHT OF TWO OVARIES OF RECIPIENTS	
				4th day	5th day	4th day	5th day
		cc.		*per cent*	*per cent*	*mgm.*	*mgm.*
AA Mixture*	$\frac{1}{16}$ cc. daily for 3 days	$\frac{3}{16}$	10	50		16†	
	1 cc. daily for 3 days	3	10	50		23	
AA 44	$\frac{1}{4}$ cc. daily for 3 days	$\frac{3}{4}$	4	0	50		23
	2 cc. daily for 3 days	6	4	25	50		35
AA 37	$\frac{1}{16}$ cc. daily for 3 days	$\frac{3}{16}$	8	100	100		28
	2 cc. daily for 3 days	6	6	66	100		34
AA 46	$\frac{1}{32}$ cc. daily for 3 days	$\frac{3}{32}$	4	75	100		17
	$\frac{1}{4}$ cc. daily for 3 days	$\frac{3}{4}$	4	25	50		22
AA 53	$\frac{1}{960}$ cc.‡ daily for 3 days	$\frac{1}{320}$	4	50		16	
	$\frac{1}{6}$ cc. daily for 3 days	$\frac{1}{2}$	12	66		46	

* The various extracts mentioned in this column represent insignificant deviations from a practically uniform method of procedure. The slightly acidified urine (green-blue to Brom Thymol Blue) is filtered and concentrated in vacuo, at 35°C. to $\frac{1}{8}$ volume and refiltered. Ethyl alcohol is added to 75 per cent. The precipitate is redissolved in water and the precipitation is repeated twice. The aqueous extract of the precipitate from the second alcohol precipitation is centrifuged to remove any material difficultly soluble in water before the third precipitation. The precipitate formed by the third alcohol treatment is dissolved in water, centrifuged, and dialized at a low temperature for 16 hours. Sometimes the aqueous solution, or the dried alcohol precipitate, has been extracted repeatedly with ether to make doubly sure of the absence of folliculin. In some of the extracts cited in the tables the alcohol precipitates were dissolved in different volumes of water so that the minimum dose of the extracts has been stated for each extract.

† The two ovaries of controls often weigh this much when autopsied at the same age, but the ovaries are quite different histologically, as is indicated by the definition of the minimal dose. The control ovary is infantile, containing only small and medium sized follicles and the uterus is threadlike and the vagina closed, while the experimental animals have ovaries showing at least a few large follicles and corpora, enlarged uterus and ruptured vaginal closing membrane.

‡ Administered as diluted solution.

were also given, an attempt being made to regulate rate of absorption by mixture with a cellular tissue. Three groups of animals were injected subcutaneously once daily for three days, one with concentrated hormone solution one with hormone mixed with ground rat testicular tissue and a third control group with testicular tissue. It was hoped the mixture with cellular tissue might decrease the rate at which the hormone became available to the tissue and hence might more closely simulate liberation from the implanted hypophysis. It was found that the ovaries of animals receiving

TABLE 3

Efficacy of single versus distributed doses

DESIGNATION OF EXTRACT	DOSAGE	TOTAL AMOUNT GIVEN	NUMBER OF CASES	RECIPIENTS SHOWING OESTROUS VAGINAL SMEAR 4TH DAY	WEIGHT OF THE TWO OVARIES 4TH DAY
		cc.		*per cent*	*mgm.*
AA 41	$\frac{1}{16}$ cc. daily for 3 days	$\frac{3}{16}$	5	20	17.6
	$\frac{1}{4}$ cc. daily for 3 days	$\frac{3}{4}$	4	50	20
	$\frac{4}{32}$ cc. 3 times daily for 2 days	$\frac{3}{4}$	5	80	20.8
AA Mixture	$\frac{1}{16}$ cc. daily for 3 days	$\frac{3}{16}$	10	50	16
	2 cc. 3 times daily for 3 days	6	10	100	37
	2 cc. daily for 4 days	8	10	50	31
	$\frac{1}{8}$ cc. 16 times daily for 4 days	$8\frac{1}{4}$	9	70	50
AA 46	$\frac{1}{32}$ cc. daily for 3 days	$\frac{3}{32}$	4	100	17
	2 cc. daily for 4 days	8	10	50	48.6
	$\frac{1}{8}$ cc. 16 times daily for 4 days, 3 hours	$8\frac{1}{4}$	9	50	54.8
AA 53	$\frac{1}{960}$ cc. daily for 3 days	$\frac{1}{320}$	6	50	16
	$\frac{1}{6}$ cc. daily for 3 days	$\frac{1}{2}$	6	33	46
AA 53 + ground rat testes	$\frac{1}{6}$ cc. + $\frac{1}{6}$ of two rat testes daily for 3 days	$\frac{1}{2}$	6	100	47

this tremendous amount of hormone, mixed with testicular tissue or given as a single daily injection of the concentrated solution, produced ovaries only three times heavier than those produced by the minimal hormone dose.

Only when a large dose of extract was given over a longer period of time did the weight of the ovaries approach that attained after moderate implant stimulation for a short time. It will be noticed (see table 4) that ovaries of young animals treated for ten days with an extract (AA 46) attained a weight of 117 mgm. or seven times the weight of ovaries stimu-

lated by the minimum dose. This ovarian weight is comparable to the heavier ovaries obtained by the implant method.

The difference in weight of the ovaries which develop as a result of the injection of extracts and implantation of rat hypophyses is primarily due to the difference in number of follicles stimulated. This difference in degree of follicular stimulation can be seen even in the ovaries resulting from the administration of minimal doses, in which case the ovaries stimulated by extracts and implants are the same weight. The ovaries resulting from the minimal dose of extract contain an average of only six corpora (average obtained from ten urine fractions—40 sets of ovaries, 19 containing corpora on the fifth day). Even the minimal dose of implant stimulates many

TABLE 4

Showing the effect of more protracted urine extract dosage on the weight of the ovaries

	DOSAGE OF EXTRACT	TOTAL DOSE	NUMBER OF CASES	RECIPIENTS SHOWING OESTROUS VAGINAL SMEAR		AVERAGE WEIGHT OF TWO OVARIES OF RECIPIENTS	
				4th day	5th day	4th day	5th day
		cc.		*per cent*	*per cent*	*mgm.*	*mgm.*
AA 42	$\frac{1}{16}$ cc. daily for 3 days	$\frac{3}{16}$	5	40	100		15.4
	$\frac{2}{3}$ cc. 3 times daily for 5 days	10	3	66	100		63
AA 46	$\frac{1}{32}$ cc. daily for 3 days	$\frac{3}{32}$	4	75	100		17
	2 cc. daily for 4 days	8	10	50	60	48.6	
	1 cc. daily for 10 days	10	6	66	83		117*
AA Mixture	$\frac{1}{16}$ cc. daily for 3 days	$\frac{3}{16}$	10	50		16	
	2 cc. daily for 4 days	8	10	50		31	

* Tenth day.

follicles to begin development and at higher doses of implants the number of follicles stimulated becomes too numerous to count easily at autopsy. On the other hand ovaries stimulated by even 32 times the minimal dose of extract average only 12 to 13 corpora (see table 5). As has been pointed out, urine extracts will, if injected over a longer period, stimulate the development of larger ovaries comparable to those stimulated by implants. When 32 times the minimal dose (1 cc. AA 46) was administered for ten days (see table 4) many follicles were stimulated, giving much larger ovaries, and so many corpora it was difficult to count them at autopsy.

Histological study of both the extract and implant cases shows that though true ovulation may be provoked by either treatment, nevertheless, with the conditions as given above, corpora lutea enclosing ova are the

prevailing feature.[4] The eggs may occupy the center of typical solid corpora or may be free in the antrum of peculiar, highly characteristic bodies which are hybrid structures between follicles and corpora. The thick cellular walls of these structures are vascularized and thus different from follicles. We may designate these luteinizing follicles as intermediate bodies. We have found although they result from all effective levels of implant doses only the higher doses of urine extract produce them in the rat. They apparently exist only when the stimulus to rapid follicular growth and luteinization are coincident. Grounds can be found for the assumption that the stimulant to follicular growth is not identical with that causing luteinization.

TABLE 5

Showing the increase in the number of corpora lutea produced by increasing dosage with urine extracts

DESIGNATION OF EXTRACT	DOSAGE	NUMBER OF CASES	AVERAGE WEIGHT OF OVARIES ON THE 5TH DAY	NUMBER OF CORPORA IN TWO OVARIES CONTAINING CORPORA BY THE 5TH DAY	AVERAGE NUMBER OF CORPORA IN THE TWO OVARIES ON 5TH DAY
			mgm.		
AA 37	$\frac{1}{16}$ cc. daily for 3 days	8	28	6, 8, 5, 6, 8, 10, 9	7
	$\frac{1}{8}$ cc. daily for 3 days	3	18	8, 6, 4	6
	$\frac{1}{2}$ cc. daily for 3 days	3	26.3	11, 16, 7	11
	2 cc. daily for 3 days	6	34.4	10, 10, 16, 11, 16	13
AA mixture	$\frac{1}{16}$ cc. daily for 3 days	10	16	6, 2	4
	1 cc. daily for 3 days	10	23	8, 5, 1, 8, 4, 8, 9, 6, 8	6
	2 cc. daily for 3 days	10	37	8, 12, 2, 11, 12, 20, 10, 14, 12, 14	12

SUMMARY

Within certain limits, the weights of ovaries stimulated to precocious development by implants of rat anterior hypophyseal tissue are roughly proportional to the amount of tissue implanted. If four times the minimal dose is given, the ovaries are increased approximately four times in weight.

On the other hand, if four times the minimal extract dose be given, the resulting ovaries are not appreciably heavier. The minimum dose of extracts of the urine of pregnant women can be increased one hundred and sixty fold and the resulting ovarian tissue in the young female is barely trebled thereby.

[4] Either treatment at a high level occasionally provokes the production of follicular or lutein cysts—a common response of the adult ovary.

This difference in the weights of ovaries that develop as a result of implant and of extract treatment is primarily due to difference in the number of follicles stimulated. The minimal effective implant treatment stimulates a much more general follicular development than does the corresponding dose of urine extracts, where a small number on "crop" of follicles are picked out by the hormone and carried to the corpora lutea stage.

ON THE PREVENTION OF CASTRATION EFFECTS IN MAMMALS BY TESTIS EXTRACT INJECTION[1]

CARL R. MOORE AND T. F. GALLAGHER

From the Department of Zoölogy and Department of Physiological Chemistry and Pharmacology, The University of Chicago

Received for publication April 24, 1929

In a group of earlier papers from these laboratories (Moore and McGee, 1928; McGee, Juhn and Domm, 1928; Gallagher, 1928) some progress was reported on the attempted isolation of the male sex hormone. It is sufficient to recall that assay methods for both the bird and mammal had been developed and that positive replacement effects had been obtained from the subcutaneous injection of lipoid extracts of bull testes. The material injected showed effects similar to those produced by the hormone from normal intact, or experimentally modified testes.

For further advancement we realized the necessity of obtaining as wide a variety and as many different objective indicators for the hormone as possible before the satisfactory refinement of preparations of the extract could be carried out. To follow the active principle of the extract through the various fractionations a suitable method of assay is imperative. Both the search for additional hormone indicators and the refinement of extractions have been actively pursued for the past three years. Sufficient success has now been attained to warrant a report of progress and in this paper, restricted entirely to the work on mammals, especial consideration will be given to the development of suitable indicators for the hormone. More detailed accounts of the various procedures are in preparation and will be published elsewhere. Similarly, the methods of extraction and of fractionation of the active material from the testis tissue will be given in more detail in later publications.

This study has been carried on as a joint project; the indicator methods were developed and the assays were made by members of the Department of Zoölogy, whereas all the chemical work on extraction and refinement was done in the Department of Physiological Chemistry and Pharmacology. It is indeed a delight to acknowledge our opportunities as due to the initiative of Professors F. R. Lillie and F. C. Koch; we have had available

[1] This investigation has been aided by a grant from the Committee on Research in Sex Problems of the National Research Council; grant administered by F. R. Lillie.

at all times their interest, council and guidance, the value of which we cannot overestimate.

Indicator methods and replacement effects. It should be emphasized that the lack of advancement in the study of the internal secretion of the testicle in the mammal has been due to a large extent to the fact that no dependable, objective, short time tests for the hormone were known. One must be able to castrate an animal and within a reasonable time determine by injection the presence or absence of the active principle before the substance or substances sought for isolation can be followed.

Using the rat and the guinea pig as the experimental animals, we have now satisfactorily developed six or more different tests, including both physiological and morphological ones. Others are being studied that give promise of real value as assay methods for testis extracts. The multiplicity of tests we consider important for the reason that it is now unknown whether the so-called "hormone" of the testis is one or several substances. Every definite and constant effect of testis removal caused by the lack of the internal secretion of the organ should be prevented in the final analysis, by injections, before one is justified in concluding that the hormone has been obtained. It is possible that one effect of castration may be prevented from developing, by injections of the testis extract, whereas a second effect may develop and persist. Such conditions would immediately raise the question of qualitative or quantitative differences—a threshold difference or a multiplicity of substances constituting the hormone. We have been and are now confronted with these questions.

The character of the tests employed by us and the success obtained in preventing the development of castration effects by injection of testicle extracts are discussed under the following headings.

A. Spermatozoön motility test (guinea pig). This test, so far employed by us only in the guinea pig, is purely a physiological indicator. It depends upon extending the life of the spermatozoön in the isolated epididymis of the normal adult animal from which the testicles have been removed. Its main limitations are 1, the extended period over which injections must be carried on for a quantitative assay, complete assays requiring approximately two months from the time of castration; and 2, the fact that it detects only the maintenance of the hormone level after castration and therefore cannot be used for the rebuilding of the castrate condition once this has developed in animals castrated for some months previous to injection. The essential facts upon which the test is based were discovered independently by Moore (1927, 1928a) and earlier by Benoit (1926). An extensive discussion and presentation of facts has been published elsewhere (Moore, 1928 a, b) and hormone replacement by injection has already been reported (Moore and McGee, 1928). Accordingly the bare facts are included here for the sake of completeness.

The removal of the testis hormone from a guinea pig by castration permits a maximal life period for spermatozoa in the isolated epididymis of 23 to 25 days (life indicated by any degree of motility when sperm is suspended in saline solution) whereas with a hormone supply from normal or cryptorchid testes the period of spermatozoön life in similarly isolated epididymides is 65 to 70 days. We have arbitrarily chosen 30 days of spermatozoön life in the isolated epididymides as a minimum positive indication of hormone effects induced by injected material. We have removed both testes from guinea pigs, injected them subcutaneously with lipoid extracts from bull testes and found that the spermatozoa within the isolated epididymides show motility 54 days after castration (Moore and McGee). This proves that our injections had almost completely replaced the testis hormone, removed through castration, so far as the limitations of this test are concerned.

B. Electric ejaculation test (guinea pig). The guinea pig seminal vesicles are tubular sacs approximately 100 mm. long and 9 mm. in diameter at the base. They are lined with a secretory epithelium that produces the seminal vesicle secretion at an approximate rate of 1.5 to 2 grams per week. The anterior lobes of the prostate gland produce a specific coagulating enzyme that when mixed with the seminal vesicle secretion, causes the formation of a firm coagulum. Thus the coagulation of the male discharge in normal matings produces the copulation (vaginal) plug. The secretory function of both the seminal vesicle and prostate gland are under the control of the internal secretion of the testicles. In castrated animals the secretory function of each is lost, but animals with one experimental cryptorchid testicle (aspermatogenetic) produce as much secretion as those with normal testes.

One can determine whether the seminal vesicles and prostate are functional without sacrificing the animal by inducing an ejaculation with an electric current through the brain; the same animal may be used many times for testing injection samples. So long as these accessory reproductive glands are in an active physiological condition and normal ejaculates are regularly obtained each week, it is proof positive that the hormone is present.

We have been able, by injecting testicular extract, to prevent the immediate loss of the secretory function of both seminal vesicles and prostate gland in adult animals following castration. Furthermore, in adult animals, castrated for a period of six months and not giving an ejaculate on electric stimulation, we have been able to induce a normal function of both seminal vesicles and prostate within a period of ten to fourteen days after the beginning of injections. The technical details of the study of this very important reaction (discovered originally by Battelli, 1922) will be published in more extensive form in the near future, as well as a study of the morphology of these glands after castration in the guinea pig.

C. Prostate cytology test (rat). Similar to the guinea-pig prostate, the rat prostate is composed of anterior, middle and posterior lobes. It has long been known that animals castrated for some months have small prostates, especially if castration was prepubertal. The normal size variations and the slow regression after adult castration, however, produce conditions that make it next to impossible to use gross size alone as a criterion of hormone action except in maximal reactions. We have made a cytological study of the three lobes of the rat prostate, using a variety of techniques, in the hope that the findings would show clear cut structural changes as a result of castration. We have found outstanding and invariable castration changes within a period of five to thirty days after testis removal. Certain cell structures, evidently dynamic centers associated with secretion, as well as the Golgi bodies, and epithelial changes permit us to diagnose a castrated animal easily within ten days after operation. In the prostate gland alone we have obtained four or five different morphological changes any one of which is adequate for hormone detection.

By injecting testicle extract subcutaneously immediately after castration, and until the animal was killed, all these morphological criteria of castration were prevented from developing. Furthermore, by injecting animals of approximately 100 days of age that had been castrated before the onset of puberty (castrated at 10 to 15 days after birth) we have built within 20 days after starting injections, a prostate gland that is entirely similar to the normal in all the cytological details that have been found subject to atrophy after castration. In so far as one is justified in basing his contentions upon these changing morphological criteria, and we believe this justified, we have effected an entire replacement of the hormone normally produced in the intact testicle by injecting testis extract. Not only have we maintained the prostate in a normal condition following testis removal but we have also literally built a functional prostate out of the undifferentiated embryonic structure that failed to develop into a secretory condition because of prepubertal castration. The preparation of material and its histological study have been carried on by Miss Dorothy Price and will be published more extensively in another place.

D. Seminal vesicle test (rat). It is well known that the size and function of the seminal vesicles of the rat are controlled by the internal secretion of the testis. Size variations and postcastrate regression in adult animals again make it unsafe to depend upon gross morphology alone as a hormone indicator, except with maximal reactions; hence, as in the case of the prostate, a cytological study has been made.

We have found readily apparent cytological changes within five to twenty days after castration, involving regression of the secretory epithelium, loss of individual and constant elements of the cells of the normal tissue and changes in Golgi bodies. On the basis of the cytology of the seminal vesicles, one can easily detect a ten day castrated animal.

By injecting adult rats subcutaneously with testis extract, beginning immediately after operation, we have prevented the development of the typical castration changes in the seminal vesicles. Animals castrated for 20 days but uninjected, have both smaller seminal vesicles than controls and smaller vesicles than they had at the time of operation. Most of the secretion contained within the seminal vesicles at the time of castration had disappeared, probably through resorption, and the vesicles themselves were of a flabby character. The injected animals, on the other hand, maintained vesicles of normal size for the duration of the injection period and when sacrificed the vesicles were as large and as thoroughly distended with secretion as in any normal male. Animals injected for a considerable period and killed several days after cessation of injection revealed the typical castrate picture. We have thus prevented the development of all the typical cytological castrate changes by injection of the testis extract. Furthermore, we have produced cytologically normal seminal vesicles in animals that were prepubertally castrated and hence had not developed the typical normal vesicle until after being injected with the extract. The gross size of such vesicles has usually been below that of the normal adult, or adults castrated and injected. The size is, however, markedly greater than in control prepubertally castrated animals that had not received injections.

Thus again we have so completely replaced the internal secretion produced by intact testicles, by means of injections, that the castrated animals cannot be distinguished from the normals either from gross size of seminal vesicles, their general appearance, secretion content, or cytological condition. The technical preparations and their study have been carried on by Miss Winifred Hughes and will be published more in detail elsewhere.

Source of injected material. The material for injection has been obtained from the lipoid fraction of bull testes. The extraction and refinement has been done by one of us (Gallagher) in the department of Physiological Chemistry and Pharmacology, and the guidance and council of Prof. F. C. Koch has been of inestimable value.

Alcoholic extracts of fresh bull testes were concentrated and shaken with equal volumes of benzene. The supernatant benzene on withdrawal serves as a "benzene stock" solution and from this different procedures have followed. In general the refinement has consisted of additional fractionations by the use of organic solvents. The general procedure is as follows: the removal of phospholipins, which constitute the main bulk of the benzene soluble material, is accomplished by precipitation with acetone after thorough removal of the benzene. The insoluble mass of phospholipin is almost totally devoid of activity.

From the acetone soluble material the cholesterol and large amounts of the neutral fats are removed by precipitation with aqueous alcohol at

low temperature. Fifty per cent alcohol has been our most common reagent. The precipitate is extracted a number of times and the soluble material concentrated and dissolved in olive oil for injection. Injections of this material are very well tolerated by mammals and in no case have we experienced the difficulty of nodule formation or open sores such as were described with earlier preparations.

Discussion. We have been forced by necessity to develop dependable indicators for the testis hormone that are of a purely objective character, sharply marked, fairly rapidly read, and capable of short time application on mammals. Our studies on the effect of castration in the rat and guinea pig have not only yielded a substantial number, but also a sufficiently diverse set of tests to lend credence to the belief that in preventing the occurrence of all the typical castration changes one has in reality injected the hormone of the testis.

The spermatozoön motility test is a valuable one but requires a rather long period for assay purposes particularly where full replacement is desired rather than mere detection of a positive reaction. The test is purely physiological and appears to involve epididymal function, which in turn is under the control of the internal secretion of the testis. In any event, and however the effect is produced, the test is capable of demonstrating the presence of at least that portion of the testis secretion which affects the spermatozoön life.

The electric ejaculation test, likewise physiological in nature, is at once a double test since for a coagulable discharge to follow stimulation both the seminal vesicles and prostate must be actively secreting. Since their secretory function is dependent upon the testis hormone, the building up of a coagulable discharge from animals castrated for some months, within a period of two weeks from the beginning of injections, is very convincing evidence that the injected extracts contain at least that portion of the testis secretion that is responsible for the maintenance of function of these accessory reproductive glands.

Passing to the rat as the experimental animal, the morphological criteria (gross as well as microscopic) invariably present in normal animals, which show pronounced changes within a few days after operation, can readily be accepted as diagnostic signs of castration. Outstanding castration changes have been found to occur in the anterior, middle and posterior lobes of the prostate, and in the seminal vesicles, as well as in the vas deferens. By injections of testis extracts we have not only prevented the castration changes from developing, but also have caused long time castrates to return to the normal condition. The work on the vas deferens up to the present time is the most incomplete phase but we believe it can be developed into an outstanding test capable of showing replacement effects on injection.

Perhaps the most impressive phase of the work is the fact that despite the diversity of tests so far employed, including as it does both morphological and physiological phases on the rat and on the guinea pig, replacement effects have been obtained for all of them with extracts prepared by the same procedure. In several of the tests the replacement effects have been complete—i.e., the castrated, injected, animals cannot be distinguished from the normal controls. With one test, the electric ejaculation test on the guinea pig in which injections followed immediately after castration, the replacement effects so far have been only partial; the postcastrate ejaculates have not been maintained up to the precastration level of the same animal. Positive effects have been obtained in that the decline in the amount of the ejaculate in castrated, injected, animals has been less precipitous than in uninjected castrates. Unfortunately, we did not employ this test with the sample that by other tests proved to be the strongest one so far employed; we believe that a threshold difference may here be involved.

Active progress is being made in tracing the active principle of the extract through various phases of refinement and at a later time we expect to present greater details of the methods of fractionation.

SUMMARY AND CONCLUSIONS

In studying the effects of castration on the rat and guinea pig we have obtained several physiological and morphological (gross and cytological) indicators of castration. Each of them alone is capable of distinguishing the castrated animal from a normal one.

By injecting fractions of a lipoid extract of bull testes into rats and guinea pigs we have not only prevented the development of the castration effects, by injecting immediately after castration, but also have been able to eliminate castration effects that have been present for some months.

The spermatozoön motility test (guinea pig), the electric ejaculation test (guinea pig), the prostate test (several different elements—rat), and the seminal vesicle test (rat), have all proved that the extracts injected were able to substitute for the internal secretion of the testicle. We have not found so far any castration change that failed to respond to the injections.

BIBLIOGRAPHY

Battelli, F. 1922. C. R. Soc. de Physique et d'histoire naturelle de Geneve, xxxix, 73.

Benoit, J. 1926. Arch. d'anat., d'hist. et d'embryologie, v, 173.

Gallagher, T. F. 1928. This Journal, lxxxvii, 447.

McGee, L., M. John and L. V. Domm. 1928. This Journal, lxxxvii, 406.

Moore, C. R. 1928a. Journ. Exper. Zoöl., l, 455.

1928b. Biol. Bull., lv, 339.

Moore, C. R. and L. C. McGee. 1928. This Journal, lxxxvii, 436.

STUDIES IN MUSCLE TONE

II. Resiliency of Muscles in Decerebrate Rigidity

LOYAL DAVIS and LEWIS J. POLLOCK

From the Departments of Neurologic Surgery and Nervous and Mental Diseases, and the Laboratory of Surgical Research, Northwestern University Medical School, Chicago, Illinois

Received for publication April 25, 1929

If a tonic labyrinthine reflex is induced in a decerebrate animal by turning the occiput down, a contraction occurs in the extensor muscles of the extremities. In a former communication (1) we pointed out that if such an intoned muscle, for example the triceps, is subjected to a stretch by gradually increasing the force, and if the stretch then is released by gradually diminishing the force, the lengthening of the muscle continues beyond the height of the mechanical extension; in fact, during the decrement of strength. The muscle begins to shorten when a small fraction of the maximum force of extension has been reached. Although at times the muscle shortens, it never returns to its original length but remains lengthened. Indeed, more often the muscle shortens but very little and frequently it remains elongated to the length reached by the mechanical extension.

Obviously this is only a record of a "lengthening reaction." This property of lengthening of the muscle could be the result of 1, fatigue; 2, a decrease in elasticity; 3, inhibition of the reflex contraction of muscle, or 4, a change in the physical property of the muscle. It was assumed that it was not the result of fatigue because of the well known and frequently described indefatigability of tonic reflexes. If it was due to diminished elasticity, although the stretched muscle might not have shortened completely with the decrement of mechanical extension, the degree of shortening which did occur would be finely graded in relation to the degree of mechanical relaxation. Contrary to this, the muscle continued to elongate after the maximum force of extension had been reached. It was found that when a muscle so intoned by a tonic labyrinthine reflex was gradually stretched a reciprocal lengthening occurred in its antagonist. This we interpreted as meaning that the lengthening was not the result of inhibition because under such a condition a reciprocal shortening should occur in the antagonist. Section of the posterior roots did not prevent the mechanically extended muscle from remaining lengthened after the removal of

the extension. Finally, if this property was the result of inhibition, the lengthening would be graded to the degree of mechanical extension and this was not the case. From these and other observations it was concluded that the property of lengthening as the result of mechanical stretch and the "pulled out" condition of the muscle was due to a change in the physical property of such a muscle so intoned by a tonic labyrinthine reflex. It is, to use the analogy suggested to us by Dr. W. T. Bovie, as if the elastic muscle is turned into a substance with properties similar to gum and that

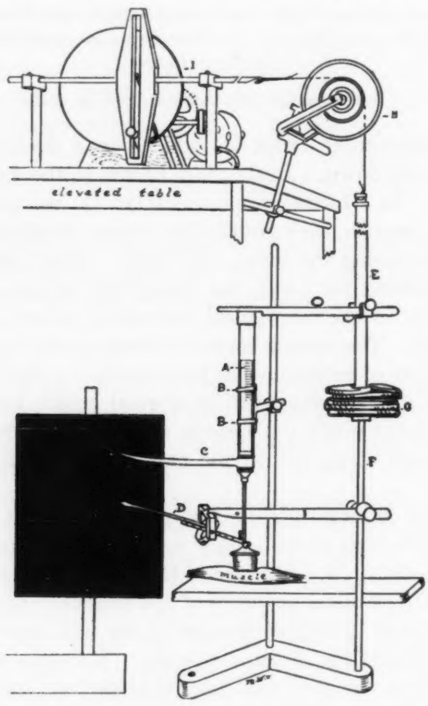


Fig. 1. Apparatus for obtaining resiliency curves

it is turned back into a substance with properties like rubber as a result of the first new reflex activity.

If a gum-like substance may be mechanically stretched and remains lengthened after the stretch is removed, it follows that if such a substance is compressed and the compression is removed it should remain indented. The following experiments then are concerned with a comparison of the resiliency curves of a normal muscle and a muscle which is intoned by a tonic labyrinthine reflex in a decerebrate animal.

Method. A sphygmometer (fig. 1—*A*), which registers up to 1000 grams was mounted upon a ring stand so that it would slide vertically within two well-fitted copper bands, *B*. A rigid writing lever, *C*, was attached to the barrel of the sphygmometer and when the latter was pressed downward it would register the distance through which the barrel was pressed. Another magnifying writing lever, *D*, was attached to the plunger of the sphygmometer to the end of which was fitted a hard rubber tip 0.5 cm. in diameter. The degree of pressure could be computed by subtracting from the distance the barrel was pressed down, the distance at

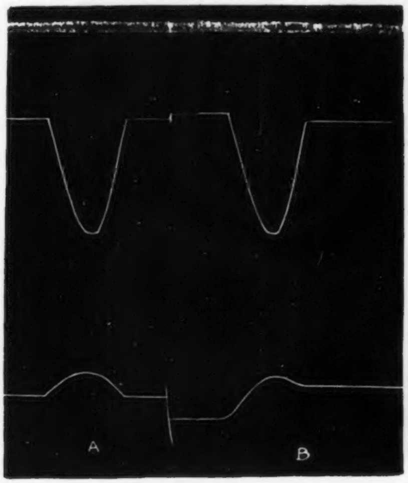


Fig. 2

Fig. 3

Fig. 2. Resiliency curves for (A) rubber cube and (B) beeswax cube. (In this and the following tracings, the upper curve is that of the force applied and the lower is the curve of resiliency.)

Fig. 3. Resiliency curve of a normal muscle.

which the plunger was pressed into the muscle and the result compared to the scale of the sphygmometer. In this particular experiment only the total force was read off on the sphygmometer scale. The barrel of the sphygmometer was subjected to downward force through a bar, *O*, attached to a metal cylinder, *E*, which moved vertically on a rod, *F*. The cylinder was properly weighted (500 to 600 grams) with small lead discs, *G*. The cylinder was lifted by means of a heavy linen cord pulled over a pulley, *H*, by a motor driven shaft geared to produce a horizontal to and fro movement at varying speeds, *I*. When the shaft moved toward the pulley, the weight upon the cylinder was transmitted through the bar to

the sphygmometer. The plunger of the sphygmometer thus compressed the muscle which was placed in a trough which could be adjusted as to height from the animal board and as to a width which would lightly constrain the sides of the muscle under examination.

Decerebrate preparations were made by the anemic method described by us in a previous paper (2). When a deafferented preparation was studied, the decerebration was performed several days later when all shock

Fig. 4

Fig. 5

Fig. 4. Resiliency curve of a muscle of a decerebrate animal intoned by a tonic labyrinthine reflex. Occiput was turned down at A and A′ but no force was applied at *A′*.

Fig. 5. Resiliency curve of a muscle of a decerebrate animal intoned by a tonic labyrinthine reflex. The posterior roots to the extremity under examination had been sectioned previously. Occiput was turned down at A.

had disappeared as was shown by the presence of good tonic neck and labyrinthine reflexes and contralateral thrusts.

When a cube of rubber is compressed and the compression is removed, a curve is obtained which shows that the deformity produced by the compression has disappeared (fig. 2—*A*). On the contrary when beeswax is so treated a deformity of indentation remains after the compression has been removed (fig. 2—*B*). A normal muscle subjected to such compression acts very much as a rubber cube and the deformity disappears when the compression force has been removed (fig. 3). When a decerebrate animal

is used, and a tonic labyrinthine reflex is induced in the muscle under examination, a contraction occurs which may be registered on the tracing. This contraction is indefatigable in a proper preparation. If the muscle is compressed under such conditions the muscle remains indented to varying degrees, but the deformation is always pronounced and often the muscle returns only to approximately the size which was present before the muscle was intoned by the labyrinthine reflex (fig. 4). This deformation could result from an inhibition of the reflex contraction induced by painful nociceptive stimuli or to a change in the physical property to the property of the muscle. If the posterior roots carrying sensation from the upper extremity have been cut in a decerebrate animal, and if the triceps intoned by a tonic labyrinthine reflex is compressed and then released, a similar curve of deformation is obtained (fig. 5). It, therefore, follows that this deformation is not the result of inhibition of the tonic reflex but is the result of a change in the physical property of the muscle so intoned by a tonic labyrinthine reflex.

CONCLUSIONS

Further proof is advanced to show that a muscle intoned by a tonic labyrinthine reflex undergoes a change in its physical properties. Not only is such a muscle similar to gum in that it may be "pulled out" and remain so, but when it is compressed and the compression then removed, it remains indented and a part or the entire amount of the bulging produced by the tonic reflex is "pressed out."

The resiliency of a muscle intoned by a tonic labyrinthine reflex is diminished in comparison to that of a normal muscle.

BIBLIOGRAPHY

(1) Pollock, L. J. and L. Davis. Arch. Neurol. and Psych. (in publication).
(2) Pollock, L. J. and L. Davis. Arch. Neurol. and Psych., 1924, xii, 288.

THE TONUS OF THE EAR MUSCLES IN THE RABBIT AFTER CUTTING THE CERVICAL SYMPATHETIC

A. LAURA HINTZE AND LLOYD SEAGER

From the Department of Physiology, University of Wisconsin Medical School

Received for publication April 27, 1929

Although most recent workers have found little or no support for the idea that the tonus of skeletal muscle is mediated by the sympathetics (see Tower and Hines, 1929; Forbes and others, 1926; Meek and Crawford, 1925), certain experiments have occasionally been described which at first glance seem to have no other possible interpretation. Such a series is that of Imagawa and Sunaga (1926) who report that after cutting the cervical sympathetic nerve in rabbits there is a decrease in the tonus of the ear muscles on the operated side, lasting anywhere from 4 to 33 days. The authors attribute recovery at this time to parasympathetic compensation since the ear again droops after the injection of atropin. This compensation breaks down when the ears are fatigued either by passive movement or by weights. The authors do not believe that vasomotor effects can explain their results because vasodilatation produced by rubbing with alcohol or by tying the ear veins has no effect. Furthermore they believe the loss of tonus outlasts the vasomotor effects following the operation.

Since drooping ears are not unusual among rabbits and since vasomotor effects can hardly be excluded without knowing the ear temperatures we have repeated the experiments just described, with especial attention to these points.

Normal rabbits frequently drop one ear while feeding and if they sit close to the pen wall the ear nearest the wall is often lowered. Occasionally animals habitually carry the ears at very different angles. Eleven normal animals with upright ears were selected and under ether anesthesia the cervical sympathetics were cut on the left side. The usual results of this procedure at once appeared, i.e., constriction of the pupil, dilatation of the ear blood vessels and a marked rise in the temperature of the ears. The ear temperatures were determined by wrapping the ear around a clinical thermometer. The position of the ear was indicated by right angle with the horizontal, 0° being horizontal and 90° upright.

In nine of our eleven rabbits, immediately on coming out of the anesthesia, the right ear returned to the erect position while the left ear remained somewhat drooped. In no case however did this drooping persist for more

than a few hours, nor did the ear ever fall forward over the eye, or hang downward with its tip lower than the base. This early lowering of the left ear might have been interpreted as a loss of tonus, had it persisted. Careful observations during the next two or three weeks, however, failed to show any real difference in the behavior of the two ears. Sometimes one ear, sometimes the other was more erect. As in the normal animal, the position of the ears seemed to be due to the various normal reflexes aroused by the different sense organs. The temperature of the ears was carefully observed until the 24th day when the experiments were terminated. During all this time the ear on the operated side remained from 32° to 36°C. The normal ear followed room temperature more closely, becoming as warm as the ear on the operated side only when room temperature became high in which case it functioned in heat dissipation.

TABLE 1

Left cervical sympathetic of a rabbit cut at 2:30 p.m.

DATE	ANGLE OF OPERATED EAR	ANGLE OF NORMAL EAR	TEMPERATURE OF OPERATED EAR	TEMPERATURE OF NORMAL EAR
July 15:				
3:30 p.m.	0	45	35	28
9:00 p.m.	15	80	36	28
July 16	75	65	35	29.5
July 18	75	75	38	38
July 19	90	90	36.5	36.5
July 23	90	90	34	29
July 24	0	90	32	28
July 26	90	0	33	33
July 29	75	75	35	34
August 1	90	90	34	33
August 4	90	90	35	26.5

Table 1 shows in condensed form the position and temperature of the ears for a typical experiment. The wide variations in the normal ear were due to different room temperatures.

Although nothing in the position of the ears justified a belief in a loss of muscular tonus, unless for the first few hours following the operation, other procedures such as Imagawa and Sunaga used were tried in the hopes of bringing some such defect to light. Rubbing the ears with ether or alcohol had no effect, an observation also noted by the Japanese workers. Likewise dry rubbing of the ears or attempts to fatigue them with weights showed no essential differences between the ears of the operated and unoperated side. If tonus in the ear muscles was being maintained because of a parasympathetic innervation this might be abolished by atropin. Accordingly each of seven operated animals received $\frac{1}{2}$ mgm. of

atropin subcutaneously. The rabbits were observed for several hours but no consistent change appeared in either ear. Three of them dropped the right ear. One dropped the left and later the right. One raised both ears from a horizontal to an upright position. The ear movements were thus varied as in any normal group of animals.

Changing the temperature of the two ears was next tried. To do this the ears were wrapped in bathing caps which had been filled either with hot water or chipped ice. In this way the ears could be brought anywhere from 14° to 45° in a few minutes. Although as may be seen from table 1, gradual increases in ear temperature due to high room temperature did not necessarily result in lowering the normal ear, this was however accomplished by sudden increases. In a large number of trials on 9 of the operated rabbits, either ear would droop when warmed to 40° or more, and either ear would rise higher if cooled to 14° or 15°. The results were most striking when one ear was warmed and the other cooled simultaneously. These heat tests served two purposes, first to find if changes in temperature were sufficient to produce any loss of tonus and second to find if the ear muscles after section of the cervical sympathetic still responded in a regular fashion to normal temperature stimuli. Since rapid heating of the ears caused them to droop it is possible that the sudden vasodilatation on section of the nerve was a factor in the temporary lowering of the ear immediately following the operation, which has already been described. On the other hand long periods of observation failed to show any consistent changes due to slow temperature changes in the environment.

SUMMARY

Rabbits after section of one cervical sympathetic showed a slight drooping of the ear on the operated side which may be interpreted as a slight loss of muscular tonus due to the vasodilation and consequent sudden rise in temperature of the ear muscles. After a few hours no difference could be distinguished between the two ears although they were observed up to 23 days.

Both ears reacted alike to rubbing with alcohol, fatigue from weights and sudden changes in temperature.

There was thus nothing disclosed either from the experiments or from long periods of observation that would justify a belief that the cervical sympathetic had any direct influence on the tonus of the ear muscles.

BIBLIOGRAPHY

Forbes, A., W. B. Cannon, J. O'Connor, A. McH. Hopkins and R. H. Miller. Arch. Surg., 1926, xii, 303.

Imagawa, T. and S. Sanaga. Zeitschr. f. d. ges. exper. Med., 1926, li, 228.

Meek, W. J. and A. S. Crawford. This Journal, 1925, lxxiv, 285.

Tower, H. and M. Hines. This Journal, 1929, lxxvii, 542.

THE PROTEIN INTAKE OF MEDICAL STUDENTS

F. P. BROOKS

From the Laboratory of Physiological Chemistry of the School of Medicine, The University of North Carolina, Chapel Hill

Received for publication April 27, 1929

In recent years papers have appeared showing the nitrogen elimination of medical students at Tulane University (1) and at Western Reserve University (2). Denis and Borgstrom showed that at Tulane the protein consumption was much below the dietary standards of the country and produced data showing this to be probably due to the effect of temperature. The results of their work did seem to show a temperature variation. Beard, at Cleveland, used data of a similar nature to substantiate the results obtained at Tulane in relation to dietary standards but showed a negative effect of a variation in average temperatures from −4.4°C. at Cleveland to 23°C. at New Orleans. The writer believed that results of similar determinations at a point intermediate between the two might be interesting insofar as it confirmed or failed to confirm these findings.

In table 1 are shown the results of analyses of 192 medical students over a period of six years on twenty-four hour urine specimens. These results are in the main those obtained by the students themselves in the regular course in Physiological Chemistry at a time when they should have been proficient in the conducting of Kjeldahl determinations. All are the average values from acceptable duplicate determinations. The completeness of the collection of the specimens was judged from creatinine values and from the specific gravity and total volume of the specimens. These results were selected from a total of 210 collections. Of these, 18 specimens were discarded because of obvious error in collection or analysis.

Of special interest are the values given for 1928 and 1929. The result for 1928 represents the average value of 225 analyses run on specimens voluntarily collected by the 35 students over a period of seven weeks. One specimen was collected each week on a different day of each succeeding week. The value for 1929 represents the average of 475 analyses of specimens collected by 35 individuals. Each student collected for a week continuously and then a different day each week for seven weeks. These analyses were run entirely by the writer and the completeness of collection was checked by volume-specific gravity relationships of the various specimens of each individual. Since the daily excretion is quite a variable

these data for 695 specimens almost have the value of specimens from that many different individuals and certainly more nearly represent the nitrogen habits of those 70 individuals than any data hitherto reported. These analyses were run in an effort to determine the effect upon protein consumption and will be considered further in another report.

These results represent the urinary nitrogen excretion of medical students eating at the University dining hall, cafeterias, boarding houses, eating clubs and private homes. The collections were made in all years except 1928 and 1929 during the month of December. In these years the work was done during January and February but, as is shown, the difference in temperature at the two periods is very slight. The temperature data were supplied by the local station of the United States Weather Bureau. Table 1 shows the results of analyses of 817 twenty-four hour

TABLE 1

Total nitrogen excretion averaged according to years

YEAR	NUMBER OF STUDENTS	AVERAGE WEIGHT	AVERAGE 24-HOUR N OUTPUT	TEMPERATURE		
				Average	Minimum	Maximum
		kgm.	*grams*	*°C.*	*°C.*	*°C.*
1916	25		10.15	6.7	−8.9	22
1925	36		10.51	5.7		
1926	27		10.80	6.5		
1927	34		10.47	7.4	2.0	12.8
1928	35	71.09	10.48	6.9	1.3	12.6
1929	35	71.61	9.64	5.0	−0.6	10.6
Average..	192	71.35	10.34			

Average weight, 825 medical students....................................69.5 kgm.
Average per 70 kgm. equivalent..........................10.43 grams N in 24 hours

urine specimens collected by 192 individuals. The daily excretions varied from 5.42 to 18.43 grams N. during a mean temperature range of 5.0 to 6.9°C. The average excretion was 10.34 grams nitrogen per twenty-four hours. This is in good agreement with the value 10.63 found at Tulane and with 11.16 as found at Western Reserve. Since the weights of the students were available only for the years 1928 and 1929 I have used the average weight of 70 North Carolina students, 400 Western Reserve students and 233 Tulane students for the purpose of calculating the 70 kgm. equivalent for Chapel Hill. This value of 69.5 kgm. shows an equivalent of 10.43 grams N per twenty-four hours. When 10 per cent is added for protein loss through fecal nitrogen the protein equivalent is 71.44 grams. This is in close agreement with the value 73.8 grams observed at Tulane.

CONCLUSIONS

A study of 817 twenty-four hour urine specimens from 192 male medical students over a period of six years shows the following results:

1. An average nitrogen excretion in the urine of 10.34 grams which corresponds with a protein consumption of 71.3 grams per 70 kgm. of body weight after adding 10 per cent for protein lost in the feces.

2. The protein consumption of medical students living at a mean temperature of 5.0 to 6.9°C. is approximately the same as found for students living at temperatures of 13.05 to 26.77 and −4.4 to −1.0°C. Hence protein habits within this range of climate and in this occupation are little affected by change of temperature.

3. The protein consumption of this class of individuals is far below the accepted dietary standards (3) as was shown by Denis and Borgstrom and by Beard. The results further support the view of Chittenden (4) that 60 grams of protein per 70 kgm. of body weight was ample to include in the diet.

BIBLIOGRAPHY

(1) Denis, W. and P. Borgstrom. Journ. Biol. Chem., 1924, lxi, 109.
(2) Beard, H. H. 1927. This Journal, lxxxii, 577.
(3) Pearl, R. 1920. The nation's food.
(4) Chittenden, R. H. The nutrition of man, pp. 226–272.

PSEUDOPREGNANCY IN THE ALBINO RAT[1]

JAMES ROLLIN SLONAKER

From the Department of Physiology, Stanford University, California

Received for publication April 29, 1929

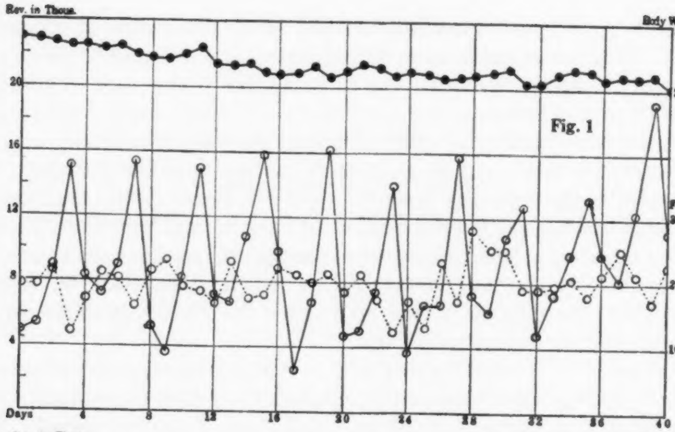
It has been demonstrated (Wang, 1923; Slonaker, 1924) that the normal female albino rat during sexual life exhibits a marked rhythm in its spontaneous activity. This is shown by decided rhythmic increases in activity which are synchronous with and indicate true oestrus. During early sexual life these spurts of activity occur each fourth day, but during later life there is usually a lengthening of the period to often as great as five-day intervals. Accompanying these oestrual phenomena the daily food consumption generally decreases. The body weight does not seem to be influenced by these oestrual cycles. It is now well established (Long and Evans, 1922; Frank, 1922; Allen, 1923; Wang, Richter and Guttmacher, 1925; Slonaker, 1927) that these rhythmic phenomena are due to the periodic activity of the ovaries and are incited by a hormone contained in the follicular fluid. Figure 1 shows typical curves of activity, food intake, and body weight of a normal female, B 2–12, between the ages of 523 and 563 days.

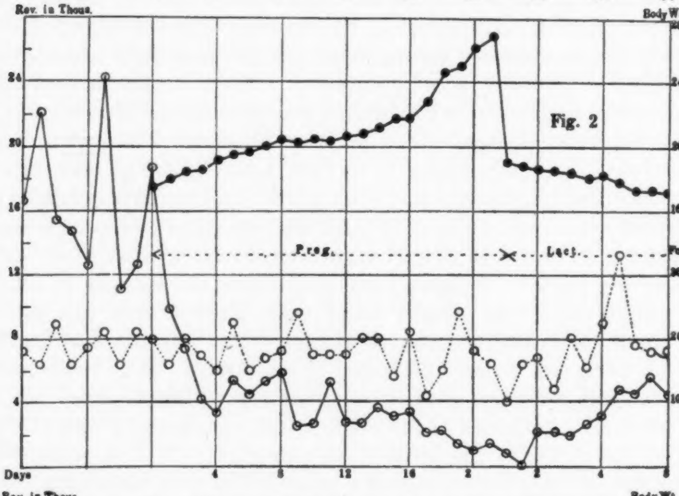
It has also been demonstrated (Slonaker, 1925) that successful copulation and pregnancy cause a noticeable change in the spontaneous activity, mammary glands, and the body weight of the animal. The rhythmic oestrual phenomena are no longer manifested and the average daily activity drops to approximately one-fourth of that previously exhibited. The mammary glands show development. The body weight increases at a gradually augmenting rate until the delivery of the young at which time the weight suddenly drops. The rate of increase in the weight and the amount of the drop at delivery depend on the number of young born. The average daily food intake, however, seems to be but slightly increased. The food energy available for the developing young is greatly enhanced by the large reduction of food energy used in activity. Figure 2 represents the curves of body weight, activity, and food consumption of a normal female (C 23–4) between the ages of 160 and 200 days, during the gestation period, and the first ten days of lactation. Occasionally true oestrus occurs during the

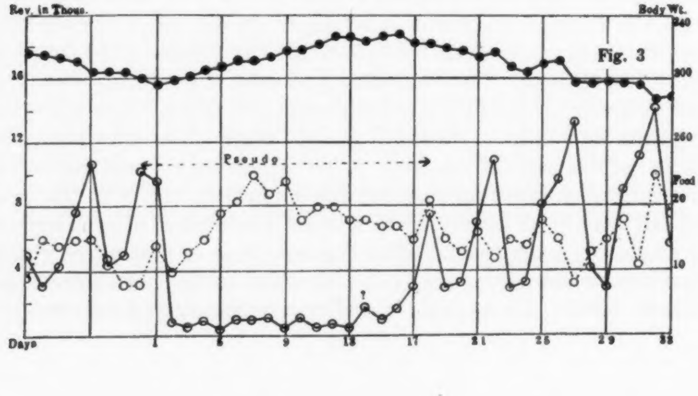
[1] This research has been conducted with the aid of the Department of Physiology and the Research Fund of Stanford University, and the Committee for Research on Sex Problems of the National Research Council.

gestation period and superfetation has been demonstrated (Slonaker, 1925). This occasional oestrus during gestation was found to occur most frequently between the twelfth and fifteenth day after coitus. During the period of normal gestation and the first ten or twenty days of lactation, in which there are usually no oestrual cycles, the ovaries show a persistence of the corpora lutea and an absence of mature Graafian follicles. This substantiates the generally accepted theory of Beard (1897) that the corpora lutea in some way are responsible for these normal reproductive activities accompanying the development of young. It has also been shown by a number of investigators (summarized by Asdell, 1928) that the development of the young is greatly interfered with if the ovaries, or all the corpora lutea, are excised during pregnancy.

After many successful copulations, as demonstrated by the presence of sperm in the vaginal smears and the characteristic drop in spontaneous activity, typical phenomena which are signs of pregnancy are noted. That is, the activity is greatly reduced, the mammary glands begin to develop, the body weight increases accompanied usually by a slight augmentation in food intake as noted in true pregnancy. These conditions continue to be manifested until between the twelvth and fifteenth day after coitus when normal rhythmic oestrual cycles are reëstablished. The resumption of oestrual rhythm is very similar to its first establishment at puberty or its reëstablishment after lactation. That is, the first oestrus is indicated by a slight pronounced spurt of activity and the succeeding cycles show rapidly increasing activities until the full magnitude is reached after a few oestrual periods have passed. Figures 3 and 4 illustrate the changes in activity, food intake, and body weight which take place during this interval. This condition is called pseudopregnancy. The phenomenon has been cited by Long and Evans (1922) and Wang (1923). Both these investigations report pseudopregnancy resulting from normal coitus and also from artificial stimulation of the cervix uteri. Long and Evans (1922, p. 80) give the results of 151 artificial stimulations. Of these trials 107, or approximately 70 per cent, caused a delay in the next cycle from 8 do 23 days. The average delay was 14.38 days. In 475 cases of pseudopregnancy under our observation we have found the average 14.53 days and a range of 7 to 19 days. They were also successful in producing pseudopregnancy by artificial stimulation in ovariectomized animals in which ovarian transplants had been successfully made. Since these transplants were made in various parts of the body where the normal nerve connections no longer existed it would appear that the suppressed action of the ovaries could not readily be attributed to a direct stimulation of such nerves as may chance to reach them. That the inhibition of oestrum is due to a corpus luteum hormone carried in the blood stream has been demonstrated by Hisaw (1929). He was able to inhibit oestrum in rats for several cycles


Rev. in Thous.
Body Wt.
Fig. 1
Food
Days


Rev. in Thous.
Body Wt.
Fig. 2
Prog.
Lact.
Food
Days


Rev. in Thous.
Body Wt.
Fig. 3
Pseudo
Food
Days

by repeated injections of extracts from blood serum of pregnant animals. When the injections were discontinued oestrum usually followed within 4 or 5 days. The vaginas of these animals during the suspension of the cycles showed the typical condition found in the rat during pregnancy and pseudopregnancy. He also found that the injection of the corpus luteum hormone was most effective when administered during or immediately following oestrum. This indicates that the follicular hormone in some manner prepares the way for the action of the corpus luteum hormone.

The prevalence of pseudopregnancy following normal coitus seems to vary with the age of the female. In a former experiment (Slonaker, 1928) in which the average of all groups of females used was considered we found that copulation was followed by pseudopregnancy between the ages of puberty and 250 days in approximately 41 per cent of the cases; between 250 and 500 days, 37 per cent; and between 500 and 700 days, 21 per cent. In a former study of the effect of early and late breeding in the oestrual cycle (Cooley and Slonaker, 1925) we found a marked difference in the prevalence of pseudopregnancy. In 72 matings made early in oestrus 33 per cent resulted in pseudopregnancy. In 65 matings made late in the cycle 57 per cent showed the characteristic pseudopregnant phenomena. This shows that the age of the female and the stage of oestrus during which coitus occurs influence the frequency of pseudopregnancies. It also substantiates the results of Hisaw, referred to above in regard to the correlation between the follicular and the corpus luteum hormones.

This investigation was undertaken with the hope of throwing some light on the underlying cause of this phenomenon. The following plans of attacking the problem were employed: 1, mating hysterectomized females with normal males; 2, mating normal females with vasectomized and castrated males; 3, autopsying normal females which showed pseudopregnancy; and 4, artificial stimulation of the cervix uteri.

1. Mating hysterectomized females with normal males. The operation on these females was not a complete hysterectomy, but consisted of removing a segment several millimeters long from each uterus about midway between the cervix and the ovary. Autopsies later showed that healing had completely closed the lumen of the separated ends of the uterus thus making it

---

Fig. 1. Curves of daily activity (solid line and open circles), food consumption (dotted line), and body weight (solid circles) of rat B 2-12 between the ages of 523 and 563 days, showing the 4 day rhythm in activity. Each high point was synchronous with oestrus.

Fig. 2. Curves of daily activity (solid line and open circles), food consumption (dotted line), and body weight (solid circles) of rat C 23-4 between the ages of 160 and 200 days showing changes during pregnancy and the first 10 days of lactation.

Fig. 3. Curves of daily activity (solid line with open circles), food consumption (dotted line), and body weight (solid circles) of rat B 2-11, between the ages of 483 and 523 days showing pseudopregnancy.

impossible for the ova to become fertilized. Since this operation did not interfere in any way with the normal oestrual cycles these females were readily mated with the normal males. In no case did pregnancy result. Of the 64 mating tests made which showed sperm in the vaginal smear or a vaginal plug 100 per cent resulted in suppressing succeeding oestrual cycles for from twelve to fifteen days following coitus. All showed typical phenomena of pseudopregnancy. Successive daily mating trials of eight hysterectomized females with normal males for a period of 87 days resulted in an almost complete suppression of oestrual cycles. The females often fought the males and at other times were non-receptive. Occasionally coitus occurred and was followed by continued suppression of oestrus. During this period

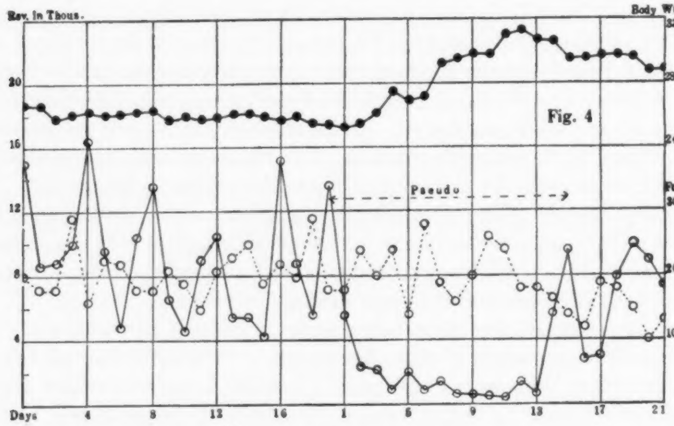


Fig. 4. Curves of daily activity (solid line with open circles), food consumption (dotted line), and body weight (solid circles) of rat B 5–13 between the ages of 593 and 663 days showing pseudopregnancy.

these females were in an almost continuous state of pseudopregnancy. After daily mating tests were discontinued normal cycles were soon reëstablished. During the interval of daily mating the activity was very irregular but greatly reduced in amount. Occasionally two or three regular cycles would appear which we attributed to lack of coitus. Was the interrupted oestrual rhythm due to the presence of sperm, the vaginal plug, or to some other cause? The fact that pseudopregnancy may result following artificial stimulation of the cervix uteri eliminates the presence of sperm or the vaginal plug as a cause of the phenomenon. This also bars the possibility of any secretion from the male being the cause.

2. Mating normal females with vasectomized and castrated males. The operation for vasectomy consisted of double ligation of the sperm ducts and

removal of the intervening portions. The removal of the testes in the rat does not prevent normal coitus for from two to four weeks following the operation. After the first coitus with either vasectomized or castrated males sperm were no longer found in the following vaginal smears. Successful coitus with either of these groups of males resulted in pseudopregnancy in both hysterectomized and normal females. In no case did pregnancy occur. From these matings 29 typical pseudopregnancies resulted. In some cases following coitus, though the average daily activity was greatly reduced similar to pseudopregnancy, there persisted slight, rhythmical fluctuations in activity which strongly suggested oestrual cycles. In one female which was mated on a high peak of 26,000 revolutions the activity was reduced to an average low daily run of 799 revolutions for a period of 44 days, before normal activity was resumed. During this interval there occurred regular four-day peaks which averaged 1600 revolutions. No mating tests nor smears were made during this time. This four-day rhythm, corresponding to that exhibited previous to coitus and continuing through into normal cycles without a break in the rhythm, indicates a regular periodic functioning of the ovaries whose normal effect on activity was partly inhibited. This inhibition was apparently initiated by the act of mating. The results of mating hysterectomized females with normal males and normal females with vasectomized and castrated males prove that the phenomena of pseudopregnancy are in no way dependent on the presence of intra-uterine young or their later reabsorption.

3. Autopsies of normal pseudopregnant females. A total of 475 pseudopregnancies has been studied. Some of these were killed on the day when the resumption of oestruation was first noted. Autopsies were made to determine the condition of the reproductive organs. The average age at which these autopsies were made was 293 days, the youngest being 120 days and the oldest 507 days. The average weight was 247 grams with a range between 204 and 310 grams. In no case was there any evidence of embryos. The uteri were more or less congested and swollen, the right having an average diameter of 4.25 mm. and the left 4.12 mm. The range in diameter for each was between 3 and 7 mm. Each of the ovaries was likewise more or less congested. The average number of corpora lutea counted in the right ovary was 3.62 with a range of from 2 to 6. The left ovary had an average of 4.5 with a range of from 3 to 7. In some cases small follicles were found and in others none could be discerned. The average number observed in the right ovary was 2; in the left ovary it was 1.4. The above conditons are such as would be found at any oestrum and present no abnormality.

4. Artificial stimulation of the cervix uteri. A number of glass probes were prepared by drawing them to different diameters and fusing the tips. Collars were placed on them at varying distances from the tip to regulate

the extent of insertion. The diameters of the tips varied from 0.7 to 4 mm. and the length from tip to collar from 9 to 30 mm. One would expect to get the greatest per cent of positive results from the probe which most nearly resembled the male organ, but such was not the case. The smallest probe gave the best results. Our results, however, were very disappointing. Following careful and gentle manipulation with the probe the large majority of the tries were negative. They were far below the per cent of positive results gotten by Long and Evans cited above. We are led to believe that the positive results that we got with the small probe may have been due to some accidental injury as well as to mere stimulation of the cervix uteri. Following normal coitus the vaginal smear is often composed almost wholly of erythrocytes which indicates an injury. In all such cases which we have observed either pregnancy or pseudopregnancy followed. It seems significant that in 18 such cases in which fertilization did not take place each resulted in pseudopregnancy. The fact that many cases of pregnancy followed indicates that the presence of erythrocytes in the smear is in no way a hindrance to normal reproduction. The results rather indicate that when pseudopregnancy occurred there was either a lack of ovulation at that particular oestrus or a failure in fertilization of such ova as were normally present.

In studying these 475 cases of pseudopregnancy we have noted a number of interesting facts. In all cases where coitus occurred very early in oestrus there was no interference with the completion of that particular cycle. Without exception the following night's activity was approximately the same as the oestrual peaks prior to coitus. This is in agreement with the results of Hisaw (1929). Following the completion of this cycle the usual phenomena of pseudopregnancy occurred. In many other cases, though the characteristic drop in activity was noted for a period of usually 3 or 4 cycles, the rhythmic activity of low order persisted. This indicates a continuance of the periodic activity of the ovaries whose normal influence on activity is in some way inhibited. Quite often frequent mating tests did not result in either pregnancy or pseudopregnancy but in a changing of the cycles from periods of 4 days to that of 5 days. In one case in which three pseudopregnancies of 15, 17 and 18 days had occurred within 55 days frequent mating tests resulted in 5-day periods for 40 days before another pseudopregnancy resulted. Later 4-day periods were reëstablished. Similar 5-day periods in a number of other animals followed mating tests. In all cases mating tests which did not result in pregnancy or pseudopregnancy greatly disturbed the regularity of the cycles, or lengthened the periods.

In figure 5 we have given the average daily changes in activity (continuous line), food intake (dotted line), and body weight (dash line) of 278 pseudopregnancies at the average age of 418 days. The

reduction in activity from 11,080 revolutions to 2750 on the third day is conspicuous. The reason this drop was not accomplished the first day after coitus was due to the fact that many matings took place early in oestrus and were followed by a completion of the cycle which resulted in high activity the night following. As can be readily seen three regular 4-day cycles were suppressed before there was any marked tendency toward increased activity. Examination of individual cases showed that the length of the period of suppressed oestruation was usually some multiple of four.

The curve of food intake shows that there was a more or less gradual increase from an average of 22.5 grams on the day of copulation (C) to 27.16 grams on the eighth day. Following this maximum it diminished

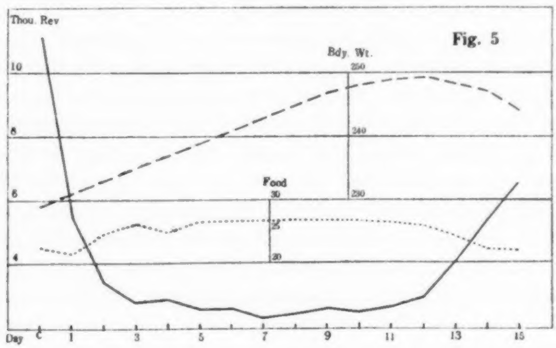


Fig. 5. Curves of averages of 278 rats between the average ages of 418 and 434 days showing the daily activity (solid line), food intake (dotted line), and body weight (dash line) during pseudopregnancy. *c*, copulation.

slightly to 27.04 on the ninth day and 26.98 grams on tenth day. During the remaining days there was a more rapid reduction to 22 grams on the fifteenth day, which was approximately normal. It is interesting to note that the marked increase in activity and the more rapid decrease in food consumption are closely associated.

The average body weight, which was 229 grams on the day of coitus, increased regularly two grams each day to 247 grams on the ninth day when the regular increase was retarded. On the tenth day the average was 248 grams; on the eleventh day it was 249 grams; and on the twelfth day the maximum average weight of 249.3 grams was reached. Following this maximum there was a noticeable decrease in average weight. The average was 248, 247, and 244 grams on the thirteenth, fourteenth and fifteenth days respectively. As can be readily seen there is a close correlation be-

tween the decrease and increase in daily activity and the increase and decrease in both the food consumption and in the body weight. The similarity of the curves of body weight and of food intake is significant. Without doubt the increase in body weight was at least partly due to the increased food intake which was greatly augmented by the use of less of its energy in activity. The decrease in body weight could be explained by the reverse of these conditions. We are unprepared to state whether any other factors are involved. Neither are we prepared to state what per cent of the increase in body weight was due to the enlargement of the reproductive organs, to the deposition of adipose tissue, or to other causes.

It is the general opinion of investigators that the mechanism which seems to be active in suppressing oestruation during pregnancy, lactation and pseudopregnancy, accompanied by enlargement of the mammary glands, involves the functioning of the corpora lutea. That the inhibition of oestrus and ovulation which follows sterile copulation and results in pseudopregnancy in the mouse and rat is associated with the prolongation of the functional life of the corpora lutea of ovulation has been verified by the work of Parkes (1929). He further states, "Since copulation is known to stimulate the luteal tissue in the normal animal, it may be supposed that copulation under certain conditions tends to bring about the luteinisation of the sterilised ovary, and that this luteinisation is responsible for the disturbance of the cycle." He does not state how this stimulation of luteal tissue is accomplished. It has been demonstrated (Evans, 1924; Evans and Simpson, 1928; Smith, 1926; Smith and Engle, 1927) that the anterior pituitary body secretes two substances which influence the activity of the ovary. One stimulates follicular maturation and the other has a luteal stimulating action. This strongly suggests that the anterior pituitary may be an intermediary agent in the process which results in continued luteal action and suppressed oestruation.

We have shown that pseudopregnancy follows successful mating of sterile males with normal females and of normal males with hysterectomized females. This together with the corroborative evidence of our autopsies, proves that the phenomenon is not due to an abortion or a reabsorption of young. It is thus associated with a lack of fertilization and implantation. Pseudopregnancy following artificial stimulation of the cervix uteri proves that it is not due to the action of any secretion from the male. The fact that 100 per cent of the non-fertile matings which showed erythrocytes in the vaginal smear after coitus, indicating injury, resulted in pseudopregnancy is very significant. However, erythrocytes were not found in all smears which were followed by pseudopregnancy. While an injury may possibly have been a contributing factor some other agency must also be involved to cause the corpora lutea and their action to persist. The fact that Long and Evans, referred to above, got pseudopregnancy in ovariecto-

mized rats in which successful ovarian transplants had been made does not suggest a direct influence of the nervous system on the corpora lutea. Taking all these results into consideration it seems most probable that the factor which is responsible for the continued action of the corpora lutea in pseudopregnancy, possibly in pregnancy also, is distributed by the blood stream in the nature of a hormone. If such a hormone exists we would most likely find its origin in the vaginal or uterine mucosa. Such a hormone could act either directly on the corpora lutea or through some intermediary agency, possibly the anterior pituitary gland. We have no evidence at present to substantiate our suggestion other than our results which eliminate other possibilities. Further investigation along this line will be necessary.

SUMMARY

1. During the first eight or ten days of pseudopregnancy there was a great reduction in spontaneous activity, an absence of oestrual rhythm, an increase in body weight, and increase in daily food intake, and a development of the mammary glands similar to that found during the same days of true pregnancy.

2. In 475 cases of pseudopregnancy the average duration from coitus to the first succeeding oestrus was 14.53 days. The shortest period was seven days and the longest nineteen days.

3. Pseudopregnancies resulted from positive matings of normal males with hysterectomized females and of vasectomized and castrated males with normal females.

4. Pseudopregnancy can be induced by artificial stimulation of the cervix uteri.

5. Autopsies made at the first oestrus following pseudopregnancy showed typical oestrual conditions of the uteri and ovaries. There was no trace of embryos or of resorption.

6. Pseudopregnancy is not caused by any secretion of the male or by young.

7. All the unfertile matings in which erythrocytes were found in the smear following coitus resulted in pseudopregnancy. Many cases, however, in which erythrocytes were not found were observed.

8. Pseudopregnancies were more prevalent in young animals and in those matings made late in oestrus.

9. Evidence indicates that the persistence of the corpora lutea is not caused by a direct nerve impulse to the ovaries.

10. There is a suggestion of a hormone secreted by the mucosa of the vagina, the uteri, or both which acts on the corpora lutea either directly or through some intermediary agent, possibly the anterior pituitary gland.

BIBLIOGRAPHY

ALLEN, E. 1923. Journn. Amer. Med. Assoc., lxxxi, 819.
ASDELL, S. A. 1928. Physiol. Reviews, viii, 313.
BEARD, J. 1897. The span of gestation and cause of birth. Jena.
COOLEY, C. L. AND J. R. SLONAKER. 1915. This Journal, lxxii, 595.
EVANS, H. M. 1924. The Harvey Society Lectures, 1923–1924.
EVANS, H. M. AND M. E. SIMPSON. 1928. Journ. Amer. Med. Assoc., xci, 1337.
FRANK, R. T. 1922. Journ. Amer. Med. Assoc., lxxviii, 181.
HISAW, F. L. 1929. Physiol. Zoölogy, ii, 59.
LONG, J. A. AND H. M. EVANS. 1922. Mem. Univ. Calif., vi, 82.
PARKES, A. S. 1929. Proc. Roy. Soc., B, civ, 171.
SLONAKER, J. R. 1924. This Journal, lxviii, 294.
1925. This Journal, lxxi, 362.
1927. This Journal, lxxxi, 325.
1928. This Journal, lxxxiv, 192.
SMITH, P. E. 1926. Proc. Soc. Exper. Biol. and Med., xxiv, 131.
SMITH, P. E. AND E. T. ENGLE. 1927. Proc. Soc. Exper. Biol. and Med., xxiv, 561.
WANG, G. H. 1923. Comp. Psychol. Mon. II, Serial no. 6, 1.
WANG, G. H., C. P. RICHTER AND A. F. GUTTMACHER. 1925. This Journal, lxxiii, 581.

THE IMPORTANCE OF THE VARIABLE CAPACITY OF THE AORTA IN AVERTING "BACK PRESSURE" IN THE RIGHT HEART AND PULMONARY ARTERY

LOUIS N. KATZ AND MORTIMER L. SIEGEL

From the Department of Physiology, Western Reserve University Medical School, Cleveland, Ohio

Received for publication April 29, 1929

The cardiodynamic effects of a constriction applied anywhere between the lower thoracic aorta and the arterioles differ from those following mitral stenosis. In the former condition no evidence exists that the pressure in the right heart and pulmonary artery increases significantly in the intact animal (Katz and Wiggers, 1927), while in the latter the proof for "back pressure" is clear as we have shown (1929). The absence of "back pressure" in constrictions of the arterioles or lower thoracic aorta is accounted for by the variable capacities of the left ventricle and of the elastic arteries above the constriction to accommodate the excess blood held back by the impediment. It is not established whether the aorta or the left ventricle is the more important physical factor which prevents "back pressure." This can be tested by producing a constriction at or near the aortic orifice. In our investigation such a stenosis was made according to the technique previously described (Katz, Ralli and Cheer, 1928). The effects of the constriction on the pressure pulses in the left auricle, pulmonary artery, and right ventricle were analyzed from photographic records made by optical means. The registration of these pressures followed the methods used in this laboratory and are briefly described in another communication (1929).

The results of previous workers who investigated the effects of aortic stenosis are incomplete and somewhat contradictory. MacCallum (1906), Gerhardt (1918), and Dzwankowska (1924), working on animals with circulation intact, observed a gradual rise in the mean pressure of the pulmonary artery following aortic stenosis; and MacCallum found no change in the right auricular mean pressure. Straub (1917), working with the heart-lung preparation, concluded that the resistance in the pulmonary arterial system was not increased, since he found a decrease in the pressure developed by the right ventricle—which, oddly enough, was associated with an increased initial pressure.

Results. *1. Immediate changes and their cause.* The results of the

present research were clear cut in showing that a constriction at the root of the aorta leads to an elevation in the pressure level of the left auricle, pulmonary artery, and right ventricle, and also to an increase in the pressure amplitude in the latter two.

The elevation in the left auricular pressure was progressive. Characteristic systolic vibrations, similar to those seen in the aortic pressure curve, appeared in the record from this chamber—indicating that the vibrations are conducted to the auricle either backward through the blood stream or directly through the adjacent walls of the aorta and auricle.

The typical changes in the right ventricular and pulmonary arterial pressure curves are illustrated in figure 1. The lower tracing shows the progressive rise of the initial pressure and pressure amplitude in the right

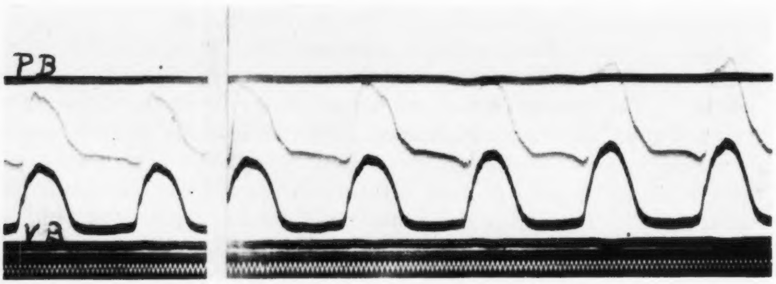


Fig. 1. Pressure curves from right ventricle (lower) and pulmonary artery (upper) showing immediate effects of stenosis at the root of the aorta (coronary sinus unobstructed). First segment control, 2nd segment the first five beats after stenosis was made. Vibrations on ventricular curve are artefacts. P.B. is pulmonary arterial base line and V.B. is ventricular base line. Time: each double vibration equals 0.02 of a second. Pulmonary arterial manometer $1\frac{1}{2}$ times as sensitive as ventricular ($\frac{1}{4}$ actual size).

ventricular curve. Contrary to Straub (1917) we found no case in which the amplitude of the curve became smaller. The ventricular curve depicts the characteristic alteration in contour of the summit, namely, a retardation of the peak toward the end of ejection, thereby changing the crest of the curve from a falling to a rising top. Similar changes in amplitude and contour are to be seen in the pulmonary arterial curve (upper tracing of fig. 1), though they are more marked since a more sensitive manometer was used. The upper curve also shows the progressive rise in systolic and diastolic pressure in the pulmonary artery. The presence of an increased pulse amplitude in the pulmonary artery, associated with an unchanged heart rate and an elevated diastolic pressure, can only mean a greater systolic discharge of the right ventricle.

This increased discharge can be caused either by an augmented return

via the coronary circuit or by damming back of blood leading to a distention of the right ventricle. An augmented coronary flow was possible since the constriction in the aorta was situated beyond the coronary openings so that the stenosis would cause a marked rise in the pressure of the aortic segment from which the coronaries emerge. But whether or not this augmented flow is great enough to explain the change in output needs further control. Straub (1917) explained the rise in initial pressure in the right ventricle on the basis of an increased coronary flow, a fact which Anrep's results (1925) seem to confirm. It does not follow that coronary flow changes are so important in the intact animal. In fact, the results of Katz and Wiggers (1927) indicate that they are considerably less when the circulation is intact.

We attempted to minimize the possibility of such an augmented coronary flow by eliminating the return of blood through the coronary sinus. In some experiments, before making the aortic stenosis, the coronary sinus was occluded by hemostat close to its opening into the right auricle; in others, it was cut and allowed to bleed freely. The first procedure may be objected to on the grounds that the obstruction of the coronary sinus leads to congestion and cyanosis of the heart. While it is true that cyanosis and congestion did appear—in fact, their appearance was used as a check on the efficiency of the occlusion—preliminary tests led to no demonstrably visible changes in cardiac activity unless the occlusion was maintained for over half an hour. At this time partial block with dropped ventricular beats appeared, the ventricles dilated, the auricles became engorged, and the beat was enfeebled. On releasing the clamp, the heart regained its normal color, size, and vigor within a few minutes. In the actual experiments, the occlusion of the coronary sinus was never maintained for more than 15 minutes, usually for no more than 5 minutes; the clamp was released temporarily between sets of observations. Furthermore, right ventricular, pulmonary arterial, and left auricular pressure curves taken after such short periods of occlusion failed to show any definite differences in contour and pressure level when compared with the control records taken during the period of interrupted coronary flow. This criticism is therefore not valid. The objection remains, however, that the congestion behind the occlusion will lead to a diversion of blood into the Thebesian vessels, some of which empty into the right heart. In order to obviate this criticism the experiments were repeated with the coronary sinus open and bleeding freely. Fortunately, the changes which follow from the continuous hemorrhage are in the opposite direction to those which "back pressure" from the stenosis would cause; and, if the experiment is made quickly, this bleeding offers no serious handicap. In the two animals in which this procedure was tried, one showed a drop in the amplitude and level of the pulmonary arterial and right ventricular

pressure curves in the record taken $\frac{1}{2}$ minute after the cut was made, the other showed no appreciable change $\frac{1}{4}$ minute after opening the coronary sinus.

Briefly stated the premises upon which these experiments were based are as follows: According to Lovatt Evans and Starling (1913), and to Anrep (1929), the coronary sinus returns 60 per cent of the total coronary inflow. However, since a large and fairly constant part of the remaining return flow enters the *left* heart *via* the Thebesian vessels, interrupting the sinus flow will eliminate somewhat more than 60 per cent of the coronary return flow to the *right* heart.

Our results with aortic stenosis show no striking differences between experiments with the coronary sinus free and intact, and those in which it was occluded or bleeding freely, except that the increase in pulse pressure

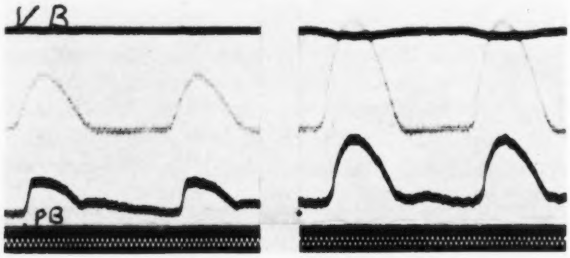


Fig. 2. Pressure curves from right ventricle (upper) and pulmonary artery (lower), showing effects of stenosis of the root of the aorta when the coronary sinus was cut and bleeding freely. Time, V.B., and P.B. same as in figure 1. Manometers of approximately equal sensitivity ($\frac{1}{4}$ actual size).

was not always present, i.e., systolic and diastolic pressures were equally elevated. A typical experiment of the more common change, i.e., an augmentation in pulse pressure, is shown in figure 2. The pressure levels and amplitude of the pulmonary arterial (lower) and right ventricular curve (upper) increase after the aortic stenosis was made even though the coronary sinus was severed.

Since identical effects were found regardless of whether the return flow *via* the coronary sinus was present or absent, it follows that "back pressure" *per se* can account for all the changes observed. The absence of a larger pulse pressure in certain experiments suggests that the changes are partly due to the augmented coronary sinus flow. In other words, Anrep's conception (1925) is only part of the story in the animal with circulation intact. The essential fact is established that the engorgement following a stenosis works back when the stenosis is so situated that the possibility

of storing the excess blood in the aorta and arteries is eliminated. The left ventricle, even with the aid of the left auricle and pulmonary veins, is therefore inadequate to accommodate the excess accumulation of blood held back by aortic stenosis. The additional capacity of the aorta is necessary in order to prevent "back pressure."

2. The secondary compensatory and other changes. The effects described in the last section are only temporary and in no way represent the conditions in a stenosis of any duration, such as clinical aortic stenosis. Compensations very quickly occur of such a nature that the unequal discharge of the two ventricles becomes equal. Otherwise, if the unequal discharge persisted, the entire blood would drain out of the systemic circuit into the pulmonic, a condition incompatible with life. Observations carried out over a period of 5 minutes show that within this short time the augmented discharge of the right side, approximately measured by pulse pressure, tends to decrease again. This is well illustrated in figure 3. The upper curve shows the primary rise of the systolic, diastolic, and pulse pressure in the pulmonary artery following the stenosis (2nd and 3rd segments), and the secondary drop two minutes later (4th segment). In this case, the changes in the diastolic pressure are not very great, but they are clearly marked in the systolic and pulse pressure. Similarly, an increase followed by a decrease of the amplitude and initial pressure level is shown in the right ventricular pressure curve (lower tracing).

The tendency on the part of the pressure in the pulmonary artery and of the discharge of the right ventricle to return to normal is accompanied by a secondary increase in the discharge of the left ventricle toward normal. Our records show that at the time that the pulse pressure and pressure levels fall again in the pulmonary artery, they gradually rise in the aorta. In fact, in mild stenosis the pulse pressure in the aorta occasionally increases temporarily beyond normal. For example, take the experiment shown in figure 4, where the stenosis was so mild as to leave the pulmonary arterial pressure unaffected. In this case, immediately following the aortic stenosis (2nd segment) there appear the characteristic changes in the aortic curve (lower tracing) such as the slow rise interrupted by a marked incisura, the systolic vibrations, and the fall in the pressure levels and pulse amplitude. Within 3 minutes (3rd segment) the pulse amplitude and pressure levels have returned approximately to normal; after another minute (4th segment) the pulse pressure is greater than normal, only to drop again one minute later (last segment).

Further proof that the compensation in the right side is accompanied by changes in the contraction of the left ventricle was obtained from a comparison of the simultaneous mean pressure changes in the pulmonary veins and superior vena cava close to the auricles (recorded with a saline U tube manometer). In table 1 are assembled the data of three successive

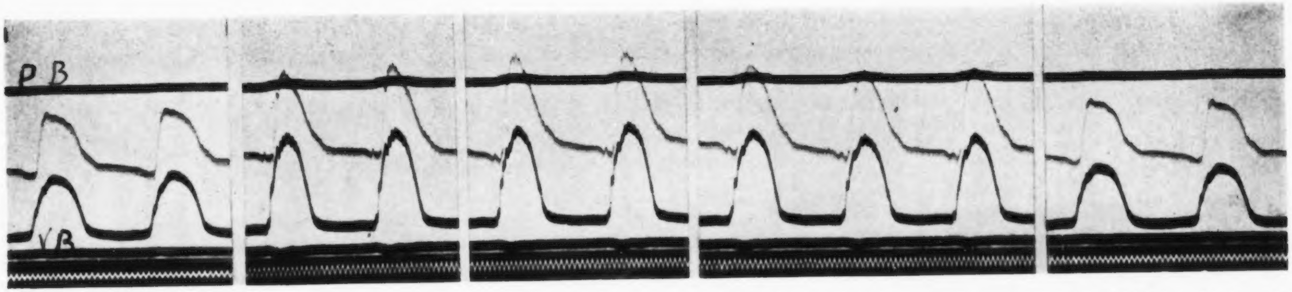


Fig. 3. Pressure curves from right ventricle (lower) and pulmonary artery (upper), showing immediate and stabilized effects of stenosis at root of aorta. (Coronary sinus unobstructed.) First and last segments controls, 2nd and 3rd segments early changes after stenosis, 4th segment secondary changes 2 minutes after stenosis made. Vibrations on ventricular curve are artefacts. Time, P.B., and V.B. as in figure 1. Pulmonary arterial manometer $1\frac{1}{2}$ times as sensitive as ventricular ($\frac{1}{4}$ actual size).

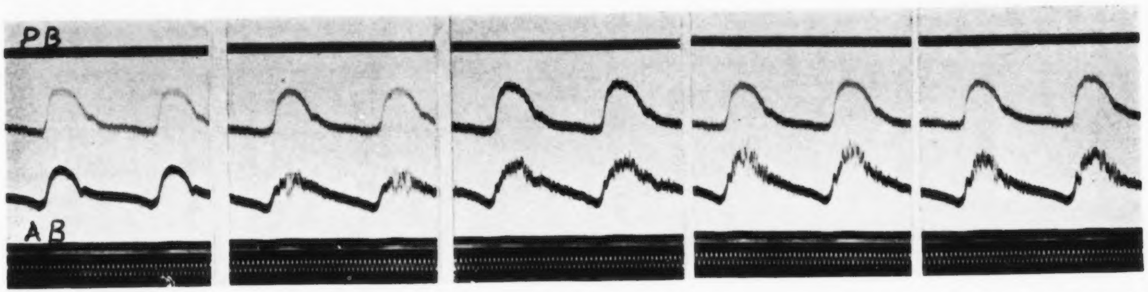


Fig. 4. Pressure curves from pulmonary artery (upper curve) and aorta (lower curve) showing effect of mild stenosis at root of aorta. First segment control, next four segments taken 1, 3, 4, and 5 minutes, respectively, after stenosis was made. Time and P.B. as figure 1. A.B. is aortic base line. Pulmonary manometer somewhat more sensitive than aortic ($\frac{1}{4}$ actual size).

experiments made on the same animal, in the first two of which the coronary sinus was intact and in the third it was occluded. The pressure in every case rises first in the pulmonary vein, and only after a lag of over a minute is it increased in the vena cava. In fact, a temporary drop appears in the vena cava pressure before the rise, due to the immediate diminution of the output of the left side which decreases the venous return to the right. The same lag occurs when compensation sets in, the pulmonary venous pressure always falls first, and this fall is reflected in the

TABLE 1

The effect of aortic stenosis on the pressures in the pulmonary veins and vena cava

EXPERIMENT KS 128	STENOSIS	MEAN VENA CAVA PRESSURE	MEAN PULMONARY VENOUS PRESSURE	CORONARY SINUS
		mm. saline	*mm. saline*	
7a	0	23	38	Unobstructed
7b	S(immediately)	23	38	
7c	S(2 minutes)	17	63	
7d	0	20	27	
8a	0	27	23	Unobstructed
8b	S(immediately)	17	93	
8c	S(2 minutes)	40	170	
8d	S(3½ minutes)	45	150	
8e	0	30	23	
9a	0	27	40	Unobstructed
9b	0	27	40	Obstructed
9c	S(immediately)	23	185	
9d	S(2 minutes)	65	150	
9e	S (2½minutes)	90	95	
9f	S(3 minutes)	27	65	
9g	S(4 minutes)	27	50	
9h	0	35	40	
9i	0	27	40	Unobstructed

S means stenosis present.

vena cava only after a minute or so.[1] The changes in the output of the left ventricle and the consequent rise in the pressure of the aorta is readily explained as the compensatory effect of the distention of the left ventricle which the aortic stenosis causes.

The augmented venous return to the right heart which results from the increased output of the left ventricle is somewhat diminished by being

[1] Incidentally the changes in series 9 are as marked as in 7 and 8 showing again that the variations in coronary flow are not significant.

stored in the systemic circuit, so that its effect is in no way comparable to the opposite effect which also follows the increased output of the left ventricle, namely, drainage[2] of the excess fluid present in the right heart and pulmonary circuit. In no other way can the return to normal of the pressures in the pulmonary artery and of the output of the right heart be readily explained. It is the *left* ventricle, then, which tends to restore the right ventricle to normal by draining away the dammed up blood in the right side.

When the degree of stenosis is very great, so that the left ventricle cannot fully compensate or fails to do so entirely, then there ensues so great a distention of the right ventricle that its contraction diminishes. Such excessive distention was particularly easy when the heart had become somewhat hypodynamic. An example of this is given in figure 5A and

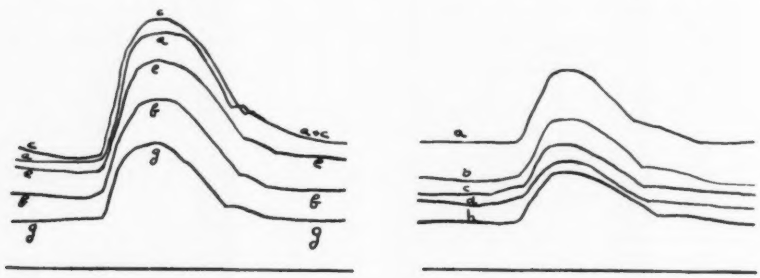


Fig. 5. Retraced pulmonary arterial pressure curves showing effect of stenosis at root of aorta on hypodynamic heart. Experiment in segment B made later than that of segment A (on same animal). In segment A curve *a* is control, curves *c*, *e*, *f*, and *g* taken 1, 3, 4, and 5 minutes, respectively, after stenosis was made. In segment B curve *a* is control, curves *b*, *c*, *d*, and *h* taken immediately, 1, 2, and 5 minutes, respectively, after stenosis was made.

B. For the sake of clarity, in these figures the pulmonary arterial curves, recorded at various times after stenosis, were retraced with base lines and onsets of ejection superimposed. In figure 5A, the curve taken one minute after a marked stenosis was made (curve *c*) shows the typical augmentation in pressure levels and pulse pressure above the normal (curve *a*). But at the end of three minutes (curve *e*) the pressures have fallen below the control and they drop still more in curves taken later (*f* and *g*). In figure 5B, a later experiment on the same animal, the pressures fall at once and continue to fall as time progresses. On inspection the heart of this animal was very markedly dilated when the records were made.

The observations detailed in this section indicate that three types of

[2] The opposite of damming up of blood.

reaction can occur in the right side of the heart following aortic stenosis, depending on the degree of stenosis and the condition of the heart: 1. The usual condition with moderate stenosis, namely, a temporary "back pressure," quickly compensated by an augmented output of the left heart.

TABLE 2

Duration of systolic phases in right heart during aortic stenosis

EXPERIMENT		STENOSIS	DURATION OF				CORONARY SINUS
			Isometric contraction	Ejection	Total systole	Cycle	
			seconds	*seconds*	*seconds*	*seconds*	
KS 121	5a	0	0.029	0.136	0.165	0.479	Unobstructed
	5b	S	0.026	0.136	0.162	0.484	
KS 121	6a	0	0.021	0.130	0.151	0.425	Unobstructed
	6c	S	0.033	0.110	0.143	0.435	
	6e	0	0.022	0.132	0.154	0.449	
KS 125	1a	0	0.048	0.147	0.195	0.565	Obstructed
	1c	S	0.035	0.151	0.186	0.586	
KS 125	2a	0	0.054	0.154	0.208	0.601	Obstructed
	2b	S	0.039	0.177	0.216	0.600	
	2d	0	0.050	0.152	0.202	0.610	
KS 129	1a	0		0.139		0.442	Unobstructed
	1b	S		0.132		0.446	
	1f	0		0.139		0.473	
KS 129	2a	0		0.146		0.473	Unobstructed
	2b	S		0.142		0.456	
	2g	0		0.145		0.461	
KS 129	3a	0		0.134		0.441	Unobstructed
	3b	S		0.133		0.429	
KS 129	4a	0		0.096		0.461	Obstructed
	4b	S		0.112		0.452	
KS 129	6a	0		0.111		0.470	Obstructed
	6b	S		0.101		0.460	

S means stenosis present.

2. Marked stenosis leading to so much "back pressure" that the right heart is distended beyond the optimum and consequently its contraction is diminished and the pressure in the pulmonary artery falls to normal or below. 3. Mild stenosis which causes an insignificant "back pressure,"

or none at all, in the pulmonary artery and right heart. The practical importance of these results is their indication that in the usual clinical case of aortic stenosis without heart failure no "back pressure" is present in the right heart and pulmonary artery.

3. Duration of the systolic phases of the right heart. The occurrence of an increased distention of the left heart, in the presence of an obstruction to outflow, caused a lengthening of total systole and ejection of the left ventricle (Katz, Ralli and Cheer, 1928). Data were, therefore, obtained in the present research to determine whether or not the immediate beats following aortic stenosis would show similar changes in the systolic phases of the right ventricle inasmuch as this ventricle is also distended and the pulmonary resistance also increased. The data are given in table 2. The figures are the average of three to five beats measured in each record. Contrary to the effect on the left side, no essential changes occurred in the duration of ejection, isometric contraction or total systole in the right side. The changes in the isometric contraction period were small and insignificant. In four experiments a shortening occurred in ejection, amounting to 0.020 of a second in KS121-6, 0.007 in KS129-1, 0.004 in KS129-2, and 0.010 in KS129-6; in two experiments (KS121-5 and KS129-3) no change occurred; in the other three a lengthening was present, amounting to 0.004 of a second in KS125-1, 0.023 in KS125-2, and 0.016 in KS129-4. The changes in total systole were even less in the four cases measured. Such differences in the changes of ejection time on the two sides make combined volume studies during stenosis of small value. The small changes in ejection may be explained on the basis of the opposing effects of the increased peripheral resistance tending to abbreviate ejection, and of the augmented distention tending to lengthen it (cf. Wiggers and Katz, 1922). One can conclude that in the right side the primary increase in discharge occurs without prolongation of ejection time.

SUMMARY

1. An analysis of the effects of stenosis at the root of the aorta on the pulmonary circuit and right heart was made on the basis of optically recorded pressure pulses from these chambers.

2. The following changes were noted immediately after a moderate stenosis of the aorta was made:

a. A progressive rise in the pressure of the left auricle and pulmonary veins and a smaller rise in the vena cava after a short lag during which the pressure fell.

b. A rise in the pulmonary arterial diastolic and systolic pressures.

c. An increased pulse pressure in the pulmonary artery.

d. A rise in initial pressure and in pressure amplitude in the right ventricle.

e. The appearance of systolic vibrations in the left auricular pressure curve.

f. A shift in the peak of the pulmonary arterial and right ventricular curve toward the latter part of ejection.

g. No significant changes in the duration of the cycle and systolic phases.

3. The effects of changes in coronary flow which follow stenosis at the root of the aorta were evaluated by comparing the results of experiments in which the coronary return flow to the right heart was free with those of experiments where it was interrupted more than 60 per cent. The coronary flow was interrupted either by occlusion of the sinus near its opening into the right auricle or by severing the sinus and allowing it to bleed freely. The merits and objections to these procedures are discussed. Except for the occasional absence of an increase in pulse pressure, no difference was noted in the two types of experiments, indicating that the changes in coronary flow play an unimportant part. The effects found are caused primarily by "back pressure" *per se*.

4. Our results indicate that moderate stenosis of the aorta leads to a damming back of blood in the pulmonary artery and right ventricle adequate in amount to raise the pressure within them and augment the discharge of the latter. The additional capacity of the aorta is necessary to accommodate the excess blood and avert "back pressure."

5. Compensatory changes follow very quickly. Evidence is given to show that they depend primarily on an increased discharge of the left ventricle which results from its increased distention with blood held back by the stenosis.

6. The effect of aortic stenosis on the right heart varies with its degree. A greater degree of stenosis is needed to produce changes in the right heart than in the left. When the degree of stenosis is excessive—the extent varying with the condition of the heart—a decrease in the pressure levels and pulse pressure eventually ensues because the right ventricle is distended beyond the physiological limit and its discharge decreased.

BIBLIOGRAPHY

Anrep, G. V. and E. Bulatao. Journ. Physiol., 1925, lx, 175.

Anrep, G. V., A. Blalock and M. Hammouda. Journ. Physiol., 1929, lxvii, 87.

Dzwankowska, H. Journ. de physiol. et path. gén., 1924, xxii, 872.

Evans, C. L. and E. H. Starling. Journ. Physiol., 1913, xlvi, 418.

Gerhardt, D. Arch. f. Exper. Path. u. Pharm., 1918, lxxxii, 122.

Katz, L. N., E. P. Ralli and S. Cheer. Journ. Clin. Investigation, 1928, v, 205.

Katz, L. N. and M. L. Siegel. (In press.)

Katz, L. N. and C. J. Wiggers. 1927. This Journal, lxxxii, 91.

MacCallum, W. G. Bull. Johns Hopkins Hosp., 1906, xvii, 251.

Straub, H. Deutsch. Arch. f. Klin. Med., 1917, cxxii, 156.

Wiggers, C. J. and L. N. Katz. 1922. This Journal, lviii, 439.

THE EFFECT OF EXCLUSION OF PANCREATIC JUICE ON GASTRIC DIGESTION

GORDON B. FAULEY AND A. C. IVY

From the Department of Physiology and Pharmacology, Northwestern University Medical School

Received for publication April 29, 1929

On a theoretical basis, the exclusion of pancreatic juice from the intestine either by ligation of pancreatic ducts or pancreatectomy should affect gastric digeston for several reasons: 1. Ivy and McIlvain (1923) found that the introduction of dilute acid into the upper intestine stimulates gastric secretion. Since in normal digestion, the acid chyme is neutralized in part by the pancreatic juice, the exclusion of the latter should lead to a more prolonged action of the acid chyme and lead to a hypernormal secretion of gastric juice. 2. It is commonly taught that acid chyme in the duodenum (Cannon, 1911) leads to an increase in the tone of the pyloric sphincter which continues until the acid chyme is neutralized. Since the pancreatic juice plays an important rôle in neutralizing the acid chyme, its exclusion should lead according to the above teaching to a hypernormal tonus of the pyloric sphincter and possibly to a gastric retention. 3. Dogs with the pancreatic ducts ligated and pancreatectomy have intestinal indigestion and polyphagia on a diet of milk, bread and meat and usually lose weight. Since polyphagia leads to an increase in hunger motility of the stomach (Carlson, 1916), it is possible that this factor increases the tonus and the cephalic or appetite secretion of the stomach and causes a more rapid emptying of the stomach.

Steinberg (1921) has observed a hypernormal secretion to occur in Pavlov pouch dogs following pancreatectomy. He explained his observations as being due primarily to a gastric retention, because the appetite secretion, or the secretion during the first hour, was not increased but decreased. He did not show, however, that a gastric retention was present.

In our work, we have studied the effect of ligation of the pancreatic ducts on gastric secretion and emptying time of the stomach, and the effect of pancreatectomy on the emptying time of the stomach. We did not repeat Steinberg's experiments on the effect of pancreatectomy on gastric secretion because of the positive character of his results.

Methods. Pavlov pouches were made on eight dogs, using our usual technique. Some two weeks or more after the operation, or after the

post-operative hypersecretion disappeared, we started the control tests, or responses to a standard meal of meat, milk and bread. From five to twenty control tests were made prior to the ligation of the pancreatic ducts.

In the experiments on emptying time of the stomach following pancreatectomy and ligation of the ducts, a test meal of 100 grams of Swift's Silver Fox food, 50 cc. milk, 45 cc. water and 35 grams of barium sulphate were fed. The time required for the stomach to empty this meal was ascertained by use of the fluoroscope. In most dogs eight control tests were made prior to the operation. All feeding was done at the same time every day.

The pancreas was removed by the usual technique and the dogs were given 10 units of insulin daily.

The pancreatic ducts were ligated doubly and cut between. Then the stumps were buried beneath the serosa of the duodenum and in the pancreas by appropriate sutures. This was done in an attempt to prevent reëstablishment of the ducts.

The gastric juice was collected for one or two hours prior to the meal and for four hours after the meal at hourly intervals. It was titrated for free and total acidity.

In some experiments sufficient alkalies were given to theoretically neutralize the gastric juice in order to determine the effect on the secretion and emptying time.

Results. *On the effects of ligation of the pancreatic ducts on gastric secretion:* Results were obtained on seven Pavlov pouch dogs, observations being made weekly for from one to six months.

It is impractical to show all the results in tables. Tables 1 and 2 show some of the results in two of the dogs. The averages are shown in table 3. Immediately after the ligation, three animals showed an immediate decrease and four an increase in gastric secretion for several days. Following this initial period, most of the animals showed a hypersecretion for three weeks after the ligation. In four, this continued until the experiment was concluded. In three, the hypersecretion decreased after about three weeks to normal and in dog VI to below normal which accounts for his low post-operative average. The hypersecretion was marked only in three of the seven dogs.

The continuous or interdigestive secretion was increased in four of the seven animals (dogs I, II, IV, and V).

The first hour's secretion was not influenced.

Atrophy of the pancreas occurred in all these dogs.

In two of these dogs, the atrophy of the pancreas was complete; in two, an exploratory operation showed reëstablishment of the ducts (which were again ligated and buried) with incomplete regeneration; in three, the ducts had not reëstablished but the pancreas was only partially

atrophied. New small ducts had probably been formed, which we were unable to find by gross dissection. In dog VI, the pancreas was on the basis of gross inspection about one-half atrophied which was the same as in dog VII. This is mentioned because of their difference in response.

Table 3 shows that the average acidity was increased definitely in four cases (dogs I, II, V, and VII).

The effect of alkalies on the gastric secretory response following pancreatic ligation. One gram of calcium carbonate was given with the meal and

TABLE 1

Dog II. Yellow

TIME BEFORE AND AFTER LIGATION OF THE DUCTS	AMOUNT SECRETED	FREE ACIDITY	TOTAL ACIDITY	REMARKS
Control before ligation				
	cc.			
December 1	22.5	0.327	0.371	Average continuous secretion 3.5 cc.
December 2	10.3	0.230	0.270	
December 4	14.0	0.230	0.300	
December 9	17.2	0.314	0.364	
December 10	29.5	0.298	0.370	
Average	18.7	0.279	0.335	
Pancreatic ducts ligated December 10th				
December 12	38.0	0.423	0.446	
December 19	36.9	0.382	0.400	Clay stools. Average continuous secretion 6.3 cc.
December 27	47.9	0.446	0.470	
January 3	41.4	0.460	0.487	
January 10	38.1	0.432	0.426	
January 17	42.5	0.473	0.491	
January 24	36.5	0.455	0.469	
January 30	38.4	0.400	0.442	
Average	39.8	0.433	0.453	

Ducts ligated December 10th, killed with chloroform on February 1st. Approximately 7½ weeks.

Autopsy showed the pancreas to be markedly atrophied.

each hour for three hours following the meal. Four of the dogs were used. On the normal animal this did not have an appreciable effect. After ligation of the ducts, it was found that in two dogs this procedure increased the secretion above the hypernormal average and in two dogs it had no effect. It was only given during the hypernormal secretory period.

The effects of ligation of pancreatic ducts on emptying of the stomach. Results were obtained on four dogs, the averages of which are shown in table 4. Detailed results on dog 11(B) are shown in table 5. The results

TABLE 2

Dog VII

TIME BEFORE AND AFTER LIGATION OF THE DUCTS	AMOUNT SECRETED	FREE ACIDITY	TOTAL ACIDITY	REMARKS
	cc.			
August 27	16.2	0.136	0.222	
August 28	16.7	0.280	0.345	
August 2	20.8	0.234	0.311	
August 3	16.3	0.327	0.414	
August 5	12.0	0.104	0.193	
August 16	18.8	0.284	0.364	
August 17	16.9	0.124	0.225	
August 19 (alkali)	17.1	0.116	0.166	
August 20 (alkali)	13.3	0.143	0.296	
August 21 (alkali)	17.1	0.181	0.202	
Average	16.5	0.192	0.264	
Pancreatic ducts ligated on September 27th				
September 3	26.0	0.352	0.384	
September 5	28.1	0.241	0.316	
September 7	28.3	0.107	0.218	
September 12	22.9	0.241	0.264	
September 15	28.2	0.225	0.334	Polyphagia
September 17	18.0	0.289	0.370	
September 18	22.1	0.318	0.371	
September 25	20.7	0.327	0.368	
October 3 (alkali)	31.4	0.239	0.341	
October 6 (alkali)	28.5	0.334	0.398	
October 13 (alkali)	31.0	0.366	0.478	
January 19	19.1	0.166	0.262	
January 20	20.2	0.159	0.255	
Average	25.0	0.320	0.335	

It was decided to perform an exploratory laparotomy to ascertain the condition of the pancreas and ducts.

Results of the operation: January 21st.

The pancreas was found to be atrophied but masses of light pink pancreatic tissue were located irregularly along the ducts denoting regeneration. There was a cyst-like cavity about 3 x 4 cm. in size extending into the body of the pancreas which opened into the duodenum. The ducts throughout the pancreas were dilated. The opening into the duodenum was closed and an attempt was made to obliterate the cystic cavity.

Results after second operation.

TIME BEFORE AND AFTER LIGATION OF THE DUCTS	AMOUNT SECRETED	FREE ACIDITY	TOTAL ACIDITY	REMARKS
	cc.			
January 31	16.6	0.316	0.368	
February 1	26.5	0.277	0.318	
February 13	28.3	0.214	0.247	
February 23	26.4	0.277	0.325	
February 25 (alkali)	19.5	0.357	0.416	
Average	25.4	0.288	0.418	

show that the emptying time was decreased in three of the four dogs, but was increased in dog 10(P), which did not develop a polyphagia following the operation, but would not eat the test meal. On the day following the operation, this particular dog showed a 22 hour gastric retention. This animal's emptying time was greater than normal for twelve days following the operation, after which it returned to normal. In dog 11(B) the emptying time was prolonged for six days after the operation after

TABLE 3

DOG	AVERAGE AMOUNT		AVERAGE FREE ACIDITY		AVERAGE TOTAL ACIDITY	
	Before	After	Before	After	Before	After
	cc.	*cc.*				
I	19.6	24.9	0.227	0.428	0.327	0.484
II	18.7	39.8	0.279	0.433	0.335	0.453
III	20.6	24.6	0.278	0.293	0.368	0.371
IV	20.8	25.8	0.309	0.328	0.395	0.419
V	41.5	46.0	0.392	0.454	0.480	0.532
VI	16.1	13.9	0.193	0.173	0.267	0.267
VII	16.5	25.0	0.192	0.320	0.264	0.335

TABLE 4

Emptying time of stomach before and after ligation of pancreatic ducts

DOG	AVERAGE 8 TESTS PRIOR TO OPERATION		AVERAGE OF TESTS TWICE WEEKLY FOR TWO MONTHS		DIFFERENCE	REMARKS
	hours	*minutes*	*hours*	*minutes*		
10(P)	4	36	5*	7	Prolonged 30 minutes	Decreased appetite
11(B)	4	24	3	34	Shortened 50 minutes	Polyphagia
12(R)	4	15	3	31	Shortened 44 minutes	Polyphagia ½ atrophy
13(T)	4	18	3	53	Shortened 25 minutes	Polyphagia

The post-operative averages do not include the results immediately after the operation, which showed an increased emptying time.

Following ligation of the pancreatic ducts dogs do better on a diet of cooked yellow corn meal, bread, and soup bone broth than on a diet of meat, bread and milk.

* For 30 days.

which it returned to below normal. In dogs 12(R) and 13(T), it was normal for three days after the operation and then was decreased. Table 5 shows the more detailed results on dog 11(B). Dog 10(P) was chloroformed and autopsied thirty days after the ligation. The pancreas was hard and fibrous, but only about one-half atrophied. Ducts were looked for, but none were found, it being believed that the functioning of minute ducts was the cause of only partial atrophy. Six weeks after ligation,

TABLE 5

Typical results on dog 11(B)—emptying time of stomach before and after ligation of pancreatic ducts

DATE (1928)	EMPTYING TIME	
	hours	*minutes*
July 7	4	10
August 6	4	5
August 7	4	30
August 8	4	30
August 9	4	45
August 13	4	35
August 14	4	35
August 20	4	30
August 29	4	35
August 30	4	30
August 31	4	30
Average	4	24
Ducts ligated on September 6, 1928		
September 7	5	38
September 8	3	40
September 9	5	15
September 10	5	20
September 12	5	45
Average	5	7
September 14	4	30
September 18	3	40
September 24	3	30
September 26	4	50
September 27	3	10
September 28	3	20
October 1	3	20
Average	3	45
October 13	2	50
October 17	3	30
October 19	4	15
October 20	3	20
October 22	3	20
October 23	4	5
October 29	3	35
October 31	3	25
November 3	3	10
November 6	3	20
November 5	3	15
Average	3	27

TABLE 6

Emptying time of stomach before and after pancreatectomy

10 units of insulin daily

DOG	AVERAGE 8 TESTS BEFORE OPERATION		AVERAGE OF TESTS AFTER OPERATION		DIFFERENCE	REMARKS
	hours	*minutes*	*hours*	*minutes*		
15(Bu)*	4	41	2	46	Decrease of 1 hour, 55 minutes	Lived 30 days; tests: 11
16(Br)	5		3		Decrease of 2 hours	Still living
17(Bo)	4	25	2	45	Decrease of 1 hour, 40 minutes	Still living
18(H)†	4	56	3	32	Decrease of 1 hour, 24 minutes	Lived 23 days; 5 tests all made 3 days prior to death†

* Chloroformed because of a marked melena-black tarry stool. Autopsy showed that bleeding must have been from mucosa of intestinal and gastric mucosa per diapedesis as there were no gross lesions.

† Died of distemper.

Dog 15 lost 7 pounds weight; dog 16, 4 pounds; dog 17, 3 pounds; dog 18, 2½ pounds.

dog 12(R) was explored and the pancreas was found to be approximately four-fifths atrophied.

The effect of pancreatectomy on the emptying time of the stomach. After making six pre-operative tests of gastric emptying time, the pancreas was removed and the emptying time followed for varying intervals as shown in table 7. Ten units of insulin were given daily to these dogs. It is seen from the average results shown in table 6 that the emptying time was decreased markedly in all cases. All the dogs had polyphagia. They all lost weight and were fed a diet of bread, milk and Silver Fox Food.

In all four of the dogs in three tests prior to the operation, 6 grams each of $NaHCO_3$ and $CaCO_3$ were mixed with the test meal. This was repeated after the operation. The alkalies had no effect on the emptying time in either case.

Dogs in which the pancreatic juice is excluded from the intestine pass bulky, clay colored stools, and usually lose weight in spite of being fed twice the normal daily ration.

Discussion. An immediate decrease in gastric secretion following ligation of the pancreatic duct is predictable, we believe, and is due to the effects of the trauma of the operation. Some increase in gastric secretion occurred in all the dogs after the recovery from the effects of the operation and continued in four of the seven until the experiment was concluded. This hypernormal secretion was marked in three of the seven dogs and the acidity was also increased. This result can only be explained as being due to the ligation of the pancreatic ducts, which results in a fibrous atrophy of the pancreas. The continuous or interdigestive secretion was also increased.

What is the cause of this hypernormal secretion? It is not due to an increase in the cephalic phase of gastric secretion because the first hour's response was not changed. It is not due to an increase in the gastric phase because a study of the emptying time of the stomach shows no retention, but a decrease in emptying time. It must be due, then, to an increase in the intestinal phase which is shown to be true, first, by the increase being more evident during the third and fourth hours after the meal, and second, by the increase in interdigestive secretion One might logically expect the polyphagia to increase the appetite secretion, but it does not. It may be that in these dogs the cephalic mechanism was being maximally stimulated prior to the operation, and that although hunger was increased after the operation, the appetite factor could not be increased. It is impossible to state the exact cause of the hypersecretion which is evidently due to an increase in the intestinal phase. Several factors must be considered: 1, increased acidity of the duodenal and upper intestinal contents; 2, intestinal indigestion with the absorption of substances acting as secretagogues; 3, and an increase in intestinal activity

due to more rapid rate of emptying the stomach, and the greater acidity. In these dogs the lower ileum and colon undergoes frequently an increase in size, which is quite marked in some.

Alkalies were given to four of the dogs during the hypernormal secretory period to determine what rôle the increased acidity of the upper intestine might be playing. In two dogs this procedure increased the secretion and in two others it had no effect. Such results do not assist any in explaining the phenomena, because we had expected the secretion to be decreased. The increase in secretion that occurred in the two dogs may have been due to alkali stimulation as observed by Boyd (1924). These results show that the lack of neutralization of the acid chyme is not a predominant factor.

TABLE 7

Dog 16 (Brownie)—emptying time of stomach before and after pancreatectomy

DATE (1928)	EMPTYING TIME	
Before pancreatectomy		
	hours	*minutes*
October 4	5	10
October 5	4	55
October 6	4	35
October 8	5	15
October 9	4	55
Average	5	
After pancreatectomy		
October 19	4	20
October 20	2	35
October 21	3	5
October 22	2	30
October 26	2	45
October 29	3	10
October 31	3	20
November 2	2	55
November 3	2	55
December 5	2	20
Average	3	*

* Period 7 weeks after pancreatectomy.

Although we were correct in expecting a hypernormal secretion of gastric juice as a result of ligation of the pancreatic ducts, we thought it would be due either to polyphagia, gastric retention, or increased acidity

of the upper intestine, which we have found not to be the causative factors. This question is still open.

Our results on the effect of ligation of the pancreatic ducts and pancreatectomy on gastric emptying time are quite definite and clear cut. They are comparable to the results that Yesko (1928) reported quite recently. This result is opposite to what we had expected and to what one might predict on the basis of the theory of the acid control of the pylorus. It should be pointed out that polyphagia was present in these animals which is probably a factor that has an effect on the motility and emptying of the stomach. It is significant that the results were more decisive in the pancreatectomized than in the ligated duct dogs, the polyphagia being greater in the former than the latter, there being no material difference in the amount of neutralization of the acid chyme. Neither did the administration of alkalies materially affect the emptying time of the stomach of the normal and pancreatectomized dogs. These results show quite decisively that the hunger or polyphagia factor or other unknown factors, are much more important in controlling the emptying of the stomach than the acid factor.

It is possible that the undigested residue in the lower ileum and the colon has some effect on the motility of these parts which reflexly increases the activity of the stomach and decreases the emptying time.

Our experience has shown that it is very difficult to cause a complete atrophy of the pancreas and to prevent reëstablishment of the ducts with the intestine.

CONCLUSIONS

1. Ligation of the pancreatic ducts in Pavlov pouch dogs results in a temporary decrease in gastric secretion lasting for a few days which is followed in most cases by a hypernormal secretion which may persist as long as six months, or may decline to normal in three or four weeks. The hypernormal secretion is due to an unexplained increase in the intestinal phase of gastric secretion. Alkalies increased the secretion in two dogs and had no effect in two others.

2. The emptying time of the stomach is decreased by ligation of the pancreatic ducts, but more so by total pancreatectomy. It is believed that hunger, or polyphagia, is the factor principally concerned in the causation of this decrease.

3. The decrease in emptying time of the stomach following ligation of the pancreatic ducts or pancreatectomy shows decisively that acidity of the duodenum as a controlling factor of the rate of emptying of the stomach is very minor in importance when compared to the hunger, or polyphagia factor, or other unknown factors.

BIBLIOGRAPHY

Boyd, T. E. 1924. This Journal, lxxi, 455.
Cannon, W. B. 1911. The mechanical factors of digestion. London.
Carlson, A. J. 1916. The control of hunger in health and disease. Chicago.
Ivy, A. C. and G. B. McIlvain. 1923. This Journal, lxvii, 124.
Steinberg, M. E. 1921. This Journal, lvi, 371.
Yesko, S. A. 1928. This Journal, lxxxv, 483.

THE MECHANISM OF OVULATION IN THE RABBIT

I. The Demonstration of a Humoral Mechanism

MAURICE HAROLD FRIEDMAN

From the Department of Physiology, University of Pennsylvania

Received for publication May 1, 1929

The rabbit differs from other laboratory rodents in that it does not ovulate spontaneously, but only after coitus. Neither artificial insemination nor injections of sperm or semen are successful in provoking ovulation (1). Yet ovulation regularly follows coitus with a vasectomized buck and can be produced by rubbing the vulva of a female in heat (2). From such considerations it is clear that if the male secretions contribute anything at all to the conditions requisite for the initiation of the process, they are not of great importance. Marshall (3) holds the view that the phenomenon is reflex in nature. In his opinion, the fact that ovulation occurs only after coitus, and then at a definite time (ten hours) afterwards, "points to the conclusion that the follicles discharge in response to a stimulus conveyed to the ovary by its nerves."

That a reflex should require ten hours for its completion seemed improbable. Nevertheless, there has been to date no decisive work to confirm or to refute this hypothesis. It was with such purpose that these experiments were undertaken.

Experimental methods and results. *A. The effect of removing the greater part of the genital tract.* Incidental to other work several investigators have removed parts of the female genital tract without preventing ovulation. Bearing in mind the importance of the cervix of the uterus in producing pseudopregnancy in the rat and mouse, and considering the possibility of intrinsic reflexes from the vagina through the uteri and tubes to the ovaries, it was decided to remove as much of these parts as possible and note the effect on ovulation.

In four rabbits, both Fallopian tubes and both uteri were removed entirely. By blunt dissection the vagina was carefully separated from the posterior wall of the bladder and was excised, leaving behind only that portion caudad to the neck of the bladder. The stump of the vaginal remainder was sutured to the anterior wall of the colon to prevent prolapse. Four weeks after the operation these females were placed with bucks and each of them accepted coitus as shown by the finding of sperm in the vaginal smear. At autopsy three days later, fresh corpora lutea, in addition

to several corpora hemorrhagica, were found in the ovaries of each of these animals. Subsequent histological examination confirmed these gross findings.

B. The fate of follicles in ovarian transplants. After the failure to prevent ovulation by the procedures employed in the first series of experiments, the possibility of the presence of some humoral mechanism was more seriously considered. Consequently, a number of ovarian transplants were made.

Through a midline incison both ovaries were entirely removed and the proximal ends of the Fallopian tubes ligated. One of the ovaries was cut into several small pieces which were immediately introduced into a scarified area in the rectus muscle through a tunnel opened by blunt dissection. This tunnel and the abdominal incision were then closed by continuous suture. After such operation, the only ovarian tissue possessed by these animals was that transplanted to the rectus muscle. After an interval of two weeks, repeated attempts were made to mate each of these females. The occurrence of coitus was determined in every case by the examination of the vaginal smear for sperm.

From five to seventeen weeks after the operation, thirteen of the fifteen females so prepared accepted coitus. In each case the animal was killed and autopsied about twenty hours after coitus.

In four animals histological examination of the transplants revealed no large follicles of any kind. In one animal a few small follicles undergoing cystic degeneration were seen. In the three other females of this group no follicular tissue of any kind could be found. Present in each of the four animals, however, were several small lobes of interstitial tissue. A thorough search for ovarian remnants or regeneration in the fatty tissues about the Fallopian stump failed to reveal the presence of ovarian tissue of any kind.

In the remaining nine of the thirteen animals accepting coitus, from one to four corpora hemorrhagica were found at the site of the transplant at autopsy. These corpora protruded from the surface of the muscle so prominently that they could be felt through the intact skin by palpation. On reflecting the skin, they projected conspicuously as large blood blisters (fig. 1). Histological examination of these blisters showed them to be typical corpora hemorrhagica with retained ova (fig. 2).

In addition to the corpora hemorrhagica, large unruptured follicles were seen in seven of the nine animals, and in two instances genuine corpora lutea were found. In one case, in which three corpora hemorrhagica and two corpora lutea occurred, the follicles had apparently ruptured into the peritoneal cavity so that no search for the discharged egg was made (fig. 3). In the other instance, however, the follicle had discharged into the surrounding tissues. In several sections ventral to the middle of the dis-

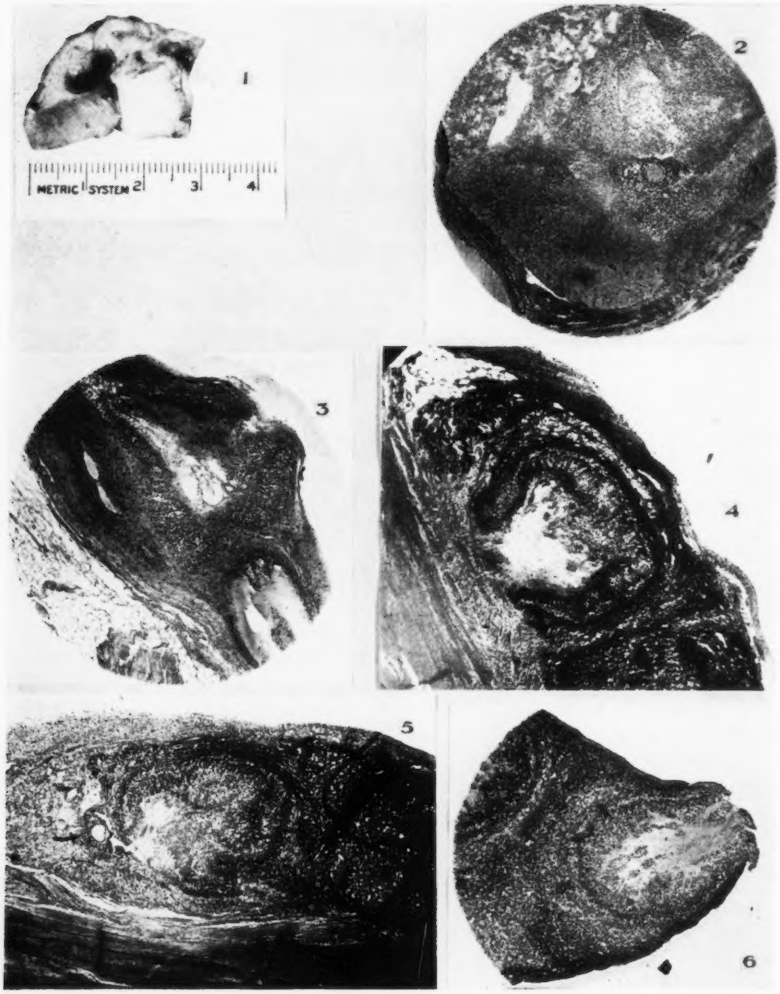


Fig. 1. Photograph of rectus muscle containing a transplant from which two corpora hemorrhagica protrude.

Fig. 2. Low power (×62) microphotograph of a section through a corpus hemorrhagicum showing the retained ovum.

Fig. 3. A low power (×62) microphotograph of a section through the transplant in animal 12 twenty hours after coitus, showing a fresh corpus luteum and a fairly large unruptured follicle; transplantation performed eleven weeks previously.

Fig. 4. Low power (×54) microphotograph of a section through the equator of a fresh corpus luteum in the transplant of animal 21, twenty-one hours after coitus; transplantation performed five weeks previously.

Fig. 5. Similar microphotograph of a section through the same corpus luteum ventral to the equator, showing the discharged egg (arrow).

Fig. 6. Low power (×62) microphotograph of a section through a fresh corpus luteum in a normal ovary nineteen hours after coitus.

charged follicle (fig. 4) the recently expelled egg could be seen (fig. 5). For comparison, a section through a normal ovary nineteen hours after coitus is shown (fig. 6) containing a corpus luteum of about the same age as those shown in the transplants.

Discussion. The fact that four females accepted coitus without the presence of large follicles or corpora hemorrhagica at the site of the transplant or in the fatty tissues about the stump of the Fallopian tube suggests that these elements are not necessary for the manifestations of heat in the female rabbit. This is in entire accord with the findings of Parkes in the mouse (4).

I have no satisfactory explanation of the high incidence of corpora hemorrhagica in the transplants. These structures are not infrequently found in the ovaries of normal rabbits after coitus. They are distinctly more numerous in the ovaries of rabbits that have not been placed with the buck until several weeks after they have come on heat; and, if the females are allowed to go through the breeding season without access to a buck, most of the ripe follicles are transformed to corpora hemorrhagica in the process of degeneration. That the corpora hemorrhagica found in the transplants represent such degenerative change is possible, but improbable, since every opportunity for coitus was allowed. It is conceivable that the high incidence of these structures in the transplants is the result of an adequate stimulus for ovulation acting on follicular tissue which is somewhat abnormal by virtue of a sub-optimal blood supply. Indeed, Heape (1) reports that any interference with the blood supply to the ovary will prevent ovulation. Furthermore Hammond (2), in commenting on the appearance of corpora hemorrhagica after coitus, concludes that in these blood follicles, "the preliminary process of ovulation has taken place (congestion of the blood vessels); but the follicle being over-mature and possibly in an early stage of absorption, rupture does not occur and the blood vessels of the theca break down into the cavity of the follicle." Yet, the fact that in some conditions these blood follicles do appear in the ovaries of females that have not had coitus, makes one hesitate to present the corpora hemorrhagica found in the transplants as evidence "that the preliminary process of ovulation has taken place."

I have no hesitation, however, in offering the corpora lutea of the transplants as evidence that ovulation did take place. From the histological picture it is clear that these corpora lutea are not the result of some lutein transformation induced by an abnormal nutrient condition. In contrast to the partially luteinized blood follicles sometimes seen in normal ovaries, and to the corpora lutea produced by the injections of extracts of the anterior lobe of the hypophysis (5), the structures in the transplants show a ruptured wall and contain no retained ovum. By comparison of the discharged follicles of the transplants with those of a normal ovary (fig. 6),

it may be noted that the former show a stage of development comparable to that attained by the discharged follicles of a normal ovary nineteen hours after coitus.

These structures are, then, genuine corpora lutea of recent origin and their presence in the transplanted ovarian tissue justifies the statement that ovulation in the rabbit can occur without the participation of the ovarian nerves. To this extent, these experiments refute the hypothesis of Marshall and demonstrate the presence of a humoral mechanism.

This obviously does not preclude the possibility that the ovarian nerves play a definite part in the process in the normal animal; and, of course, it does not exclude the possibility that the process is initiated by afferent impulses from the sensory nerves of the vagina. Yet, if such impulses are of prime importance they must come from the small portion of the vagina between the neck of the bladder and the external orifice, inasmuch as the whole of the genital tract cephalad to this portion can be removed without preventing ovulation.

SUMMARY

1. Removal of the whole of the female genital tract, with the exception of that portion of the vagina between the neck of the bladder and the external orifice, does not prevent ovulation in the rabbit.
2. Ovulation may occur in transplanted ovarian tissue.
3. Up to about ten hours after ovulation, the changes observed in the discharged follicles of the transplants simulate those seen in the discharged follicles of the normal ovary.
4. These results indicate that following coitus there occurs in the humors of the female rabbit some change, or changes, capable of provoking ovulation.

BIBLIOGRAPHY

(1) Heape: Proc. Royal Soc., 1905, lxxvi B, 260.
(2) Hammond and Marshall: Reproduction in the rabbit. Oliver & Boyd, Edinburgh, 1925.
(3) Marshall: The physiology of reproduction. Longmans, Green & Co., 1922, 561.
(4) Parkes: Proc. Royal Soc., 1926, c (B), 172.
(5) Parkes: Proc. Royal Soc., 1929, civ (B), 189.

AN ELECTRON TUBE STIMULATING DEVICE

An Adaptation of the Intermittent Valve Oscillator

C. J. CAMPBELL

From the Physiological Laboratory, Syracuse University, College of Medicine

Received for publication April 20, 1929

The following is a brief description of a special adaptation of the intermittent valve oscillator described by Taylor (1926, 1927). Similar behavior of oscillating vacuum tubes has frequently been observed by radio amateurs as a result of accidental imperfections in the "grid leak" of the oscillator circuit. The principle involved is in brief this: if the "grid leak" resistance of a valve oscillator is sufficiently high, a negative charge will accumulate on the grid and sustained oscillation will cease, and intermittent oscillation will take place. In other words, a period of oscillation will be followed by a period of non-oscillation and this in turn by another period of oscillation, and so on. The duration of the periods and the rate at which they occur is dependent upon the capacity in the grid circuit and the value of the high resistance leak between grid and filament (i.e., the rate at which the negative charge on the grid accumulates and leaks off), and also upon the constants of the oscillating circuit proper.

Thus in a circuit arranged for intermittent oscillation, the mean grid potential is changing periodically. This implies a periodic change in the value of the direct current flowing between plate and filament of the tube. If the various constants of the circuit are proper, there is in the plate circuit then a pulsating unidirectional current. A fraction of this pulsating current may be led off from the plate circuit and used for repetitive stimulation of irritable tissues.

The frequency of stimulation may be controlled by varying the time constant of the grid circuit. The intensity of stimulation may be varied by inserting a potentiometer in the plate circuit and leading from one arm of the potentiometer to the stimulating electrodes.

The first circuit tried was that of figure 1. Any of the ordinary three electrode tubes will operate satisfactorily. The frequency of stimulation is controlled by changing the value of the capacity in the grid circuit and the value of the resistance R. The smaller the resistance R and the capacity Cg, the higher the frequency. The capacities shown are those of condensers which happened to be on hand. Below a value $R = 1 \times 10^6\omega$, intermittent oscillation ceases. The frequency of stimulation obtainable

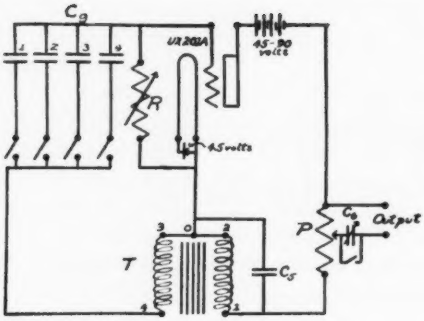


Fig. 1

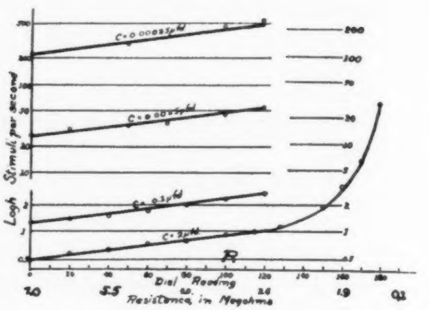


Fig. 2

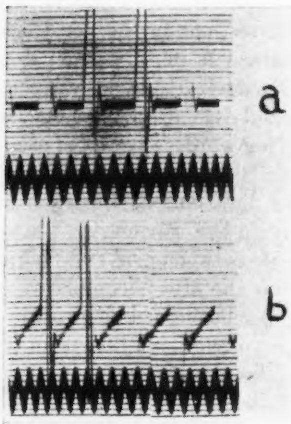


Fig. 3

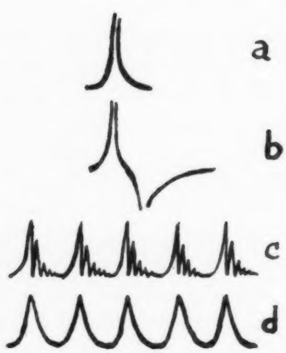


Fig. 4

Fig. 1. June 8, 1928. *Intermittent Oscillator*, $Cg_1 = 2\mu$fd. $Cg_2 = 0.1\mu$fd. $Cg_3 = 0.0025\mu$fd. $Cg_4 = 0.00025\ \mu$fd. R is a variable non-inductive grid leak of from 0.1 to 7 megohms; T, an audio-frequency transformer; P, a 2,000 ohm potentiometer. $C_5 = 0.0025\mu$fd. $C_6 = 0.001$ to 0.5μfd.

Fig. 2. The hyperbolic logarithm of frequency of stimuli plotted against the grid resistance R. Actual numerical values of frequency are indicated on the ordinate. The sharply inflected portion of the upper three curves is omitted to emphasize the fact that the linear portions of the curves are parallel.

Fig. 3. June 21, 1928. Output of stimulator recorded c̄ string galvanometer retouched. $Cg = 2\mu$fd. $R = 1.5$ megohm. a, with $C_6 = 0.0025\mu$fd. b, with C_6 short circuited. Time marker 100 d.v. per second.

Fig. 4. Diagram of output wave of stimulator as sketched from standing waves on cathode ray oscillograph. a, $Cg = 0.0025\mu$fd. $C_6 = 0.0025\mu$fd. b, $Cg = 2.0\mu$fd. $C_6 = 0.0025\mu$fd. c, as reported by Gilson with C_5 in the circuit. d, as reported by Gilson with C_5 removed.

with this particular arrangement is from 1 shock in 3 seconds to about 5,000 per second. The manner in which frequency varies with changes in R is shown in figure 2.

Increasing the capacitance C_5 increases the frequency of stimulation (cf. Taylor, 1927). The condenser C_6 acts as a simple high pass filter and removes from the output any constant current and any undesirable slow component of the pulsating current. The smaller values of C_6 may be used in stimulating medullated nerve and the larger values when working with non-medullated nerve and cardiac muscle. The general form of the output wave is shown in figure 3.

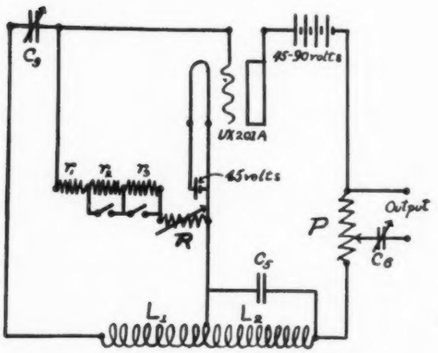


Fig. 5

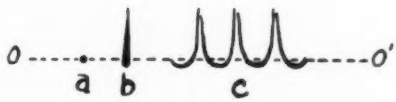


Fig. 6

Fig. 5. October 10, 1928. Diagram of circuit of stimulator using a radio frequency oscillator. $Cg = 0.00025$ to 0.01μfd. $r_1 = 3$ megohm fixed grid leak; $r_2 = 5$ megohms; $r_3 = 10$ megohms. R, P, and C_5 and C_6 as in figure 1. L_1L_2 made of 80 turns of no. 26 double silk covered wire on a 4 inch cylindrical form and tapped at the 20th turn for the filament return.

Fig. 6. Diagram of traces of output on cathode ray oscillograph. a, size of spot when x and y plates are short circuited. b, trace generated with stimulator output on y plates; x plates short circuited. c, trace generated with stimulator output on y plates and spreading device connected to x plates. OO′ zero axis.

This form of stimulator has been used by Knowlton (in preparation) to study the reciprocal inhibition in the claw of the crab, and was considered fairly satisfactory. Gilson (1928), in using this stimulator found it necessary to remove the potentiometer P from the position shown in figure 1 and to insert it between the terminals *0* and *2* of the audio frequency transformer. This reduced the stimulus artifact when recording action currents. He also found that the output as examined with the cathode ray tube was oscillatory in character rather than pulsating (fig. 4c). Removal of C_5 gave a proper shape to the output (fig. 4d). The distributed capacity

of the transformer windings sufficed to maintain oscillation. The modified apparatus is used also as an audiometer.

In this laboratory examination of the output of the stimulator by means of the cathode ray oscillograph gave the records shown in figure 4a, b. When the capacity Cg is large the shape of the stimulating current is complex. The differences in the output of Gilson's apparatus and our own are due probably to differences in the electrical constants of the iron cored, audio-frequency transformers used, and to the changed position of the potentiometer in his circuit.

To simplify the apparatus and to avoid complicated wave forms the circuit shown in figure 5 was adopted. The oscillating circuit here operates

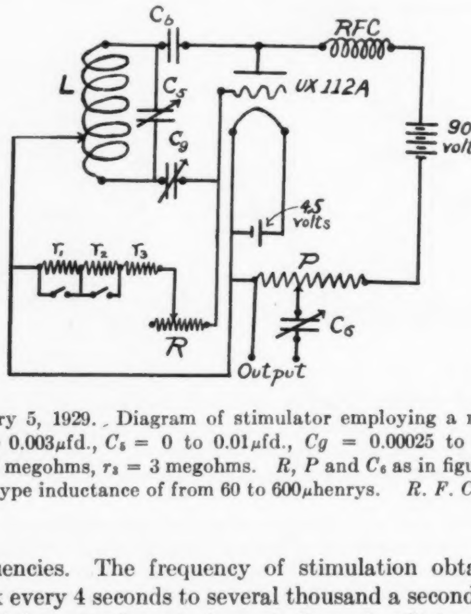


Fig. 7. January 5, 1929. Diagram of stimulator employing a radio frequency oscillator. $C_b = 0.003\mu$fd., $C_5 = 0$ to 0.01μfd., $Cg = 0.00025$ to 0.1μfd. $r_1 = 10$ megohms, $r_2 = 5$ megohms, $r_3 = 3$ megohms. R, P and C_6 as in figures 1 and 5. L is a variometer type inductance of from 60 to 600μhenrys. *R. F. C.* is a radio frequency choke.

at radio frequencies. The frequency of stimulation obtainable varies from one shock every 4 seconds to several thousand a second. A diagram of the output of this stimulator is shown in figure 6. String galvanometer records taken by Forbes (1928) with a similar piece of apparatus show a definitely diphasic wave. He states, however, that the electrical response of nerve to a single shock is never repetitive.

In order to separate the radio frequency, audio-frequency and direct current branches of the circuit, the arrangement of figure 7 was adopted. This is the usual Hartley oscillator of radio practice with the addition of the variable, high resistance leak R and the additional fixed resistances r_1, r_2, and r_3. With this apparatus one may obtain rates of stimulation varying from one shock a minute or less up to several thousand shocks per

TABLE 1

FILAMENT VOLTAGE	FREQUENCY SHOCKS PER MINUTE	REMARKS
2.5	66.0	Cg = 0.1 μ fd. Plate voltage = 90. Grid resistance = 9.5 megohms. C_s = 250 μμ fd
3.0	45.0	
3.5	44.5	
4.0	44.0	
4.5	44.0	
5.0	44.0	

TABLE 2

PLATE BATTERY VOLTAGE	FREQUENCY SHOCKS PER MINUTE	REMARKS
22.5	58	Filament voltage = 3.7. Grid resistance = 9.5 megohms. Cg = 0.1 μ fd. C_s = 250 μμ fd
45.0	48	
67.5	44	
90.0	42	
112.5	42	
135.0	42	

TABLE 3

C_s μfds	FREQUENCY SHOCKS PER MINUTE	REMARKS
0	49	Cg = 0.02025 μ fd. Plate battery = 90 volts. Filament voltage = 3.7. Grid resistance = 37 megohms
0.00025	52	
0.00125	58	
0.00225	61	
0.00325	64	
0.00425	68	
0.00925	88	
0.01025	360	

TABLE 4

ROTATION	FREQUENCY SHOCKS PER MINUTE	REMARKS
degrees		
0	52	Cg = 0.02025 μ fd. C_s = 0.00025 μ fd. Filament voltage = 3.7. Plate battery = 90 volts
45	52	
90	56	
120	84	
158	136	
180	0	
213	130	
270	50	
360	52	

second. The variation in rates with changes in grid capacity and grid leak resistance is similar to that shown in figure 2. The hyperbolic logarithm of the frequency of stimulation is a linear function of the resistance until the resistance becomes smaller than 3 megohms. At this point there is a sharp inflection of the curve. On this account the resistance r_3 of 3 megohms is always left in the circuit and only the linear portion of the logh frequency—grid resistance curve is utilized.

The shape of the output wave is that of figure 6c except that none of the wave is below the base line until the grid capacitance C_5 exceeds a value of about 0.1 μ fd. At this point the wave form becomes complex and diphasic.

Other factors than the grid capacitance and resistance affect the rate of stimulation. Ordinarily it is convenient to keep these other factors constant and vary the grid circuit only.

A. The effect of *change of filament temperature* may be seen from table 1.

B. The effect of *change in plate battery voltage* is illustrated in table 2.

C. The effect of *change in the tuning capacitance* C_5 is shown in table 3. From $C_5 = 0.01025\ \mu$ fd. on to $C_5 = 0.01425\ \mu$ fd. the frequency increases rapidly and then diminishes rapidly until at $C_5 = 0.01925\ \mu$ fd the frequency is 420 per minute. With tuning capacitances greater than this last value the behavior of the oscillator is discontinuous and unstable.

D. The effect of *changes in value of the inductance* L is shown in table 4. L is a variable inductance of the "variometer" type and the value of L is changed by rotating the inner coil in a clockwise direction.

Inspection of these tables shows the advisability of controlling the rate of stimulation by altering the time constant of the grid circuit after first deciding on proper values for the oscillating circuit LC_5.

The stimulation intensity increases somewhat as the capacitance of Cg is increased.

Further work is being done to determine by means of the cathode ray oscillograph the circuit characteristics which give the best wave form in the output, and to measure accurately the changes in intensity and duration which accompany changes in rate.

SUMMARY

A device utilizing an oscillating electron tube and containing no moving mechanical parts other than a few simple electrical switches and sliding contacts, which is capable of delivering electrical stimuli at rates varying between a few shocks per minute to several thousand per second is described in brief. Further work is being done to determine the best form of the apparatus and to make accurate measurements of the output.

BIBLIOGRAPHY

Forbes, A. 1928. Personal communication.
Gilson, A. S. 1928. Personal communication.
Taylor, L. S. 1926. Journ. Opt. Soc. Amer. and R. S. I., xii, 149.
1927. Journ. Frank. Inst., cciii, 351.

STUDIES IN HUMAN PHYSIOLOGY

III. Alveolar Air and Blood Gas Capacity

FRED R. GRIFFITH, Jr., GEO. W. PUCHER, KATHERINE A. BROWNELL, JENNIE D. KLEIN and MABLE E. CARMER

From the Department of Physiology, University of Buffalo, Buffalo, N. Y.

Received for publication February 15, 1929

In preceding reports we have presented data regarding the intra-individual variations of oral temperature and basal metabolism (1) and of systolic blood pressure and pulse rate (2) of five subjects who were under observation during the years 1925 and 1926. This installment of the series records the composition of the alveolar air and the blood gas capacity of the same individuals. The present observations were made in conjunction with, and under the same general conditions as those previously reported.

Collection of the blood sample: this was always taken from an arm vein; 15 to 20 cc. were taken each time, because, in addition to the blood gas analysis, a rather complete chemical analysis, which will be reported upon later, was also made. The sample was transferred immediately to a small bottle containing sufficient oxalate, shaken thoroughly and placed at once in the ice-box.

During the first year (1925) the sample was taken either just before or just after completion of the metabolism determination; during the second year it was, with very few exceptions, taken only after completion of the collection of the expired air; in either case it was taken only after the body had attained a strictly basal state.

Samples were taken approximately once a week; that is, during 1925, they were taken in conjunction with practically every metabolism determination; during 1926, when the metabolism determinations were done on an average twice a week, the blood sample was taken in connection with only every other determination.

These data are derived from only four of the subjects; K.L., the woman subject, during 1926 had no blood samples taken. In 1925, then, we have the data of the two men, A.B., C.D., and the two women, E.F., and G.H.; during 1926, only those of the two men.

Determination of the blood gas capacity: The blood was used for this purpose within half an hour to two hours of the time of its collection, having been kept on ice in the meantime. A little over one cubic centimeter was

placed in a 250 cc. separatory funnel, used as a tonometer; this was then filled with air which was obtained as follows:

The experimenter, who had been engaged in ordinary laboratory work for at least an hour or two, sat down and rested quietly for five minutes; during the last of the five minutes he timed his respiration so as to complete an inspiration and expiration every five seconds. This was all done so that a standard condition as to rate of carbon dioxid production and elimination might be obtained which would be comparable from day to day.

At the the end of this preliminary five minutes the experimenter expired as deeply as possible to the room and immediately inspired to the limit of a normal inspiration from a bag containing pure oxygen. He then transferred to an empty bag and rebreathed at the rate of a complete respiration every five seconds for thirty seconds, making, at the end of this time, the deepest possible expiration into the previously empty bag. This bag then contained a gas of practically alveolar carbon dioxid tension, but rich in oxygen. This is merely an adaptation of the Plesch method of obtaining alveolar air (3) but with complete disregard of the true alveolar oxygen content. And whether or not this method gives the true alveolar carbon dioxide tension is immaterial for the present purpose, since all that was wanted was a gas of approximately this carbon dioxid content but which before all else should be reasonably constant and standard in composition from day to day. The enrichment with oxygen was for the purpose of providing sufficient oxygen pressure to ensure complete oxygen saturation of the blood.

This gas was used to fill the tonometer; equilibration was carried out at room temperature with the use of a motor-driven rotator for 20 to 30 minutes; during this time the tonometer was repeatedly flushed out with fresh samples of the gas. At the end of this time one cubic centimeter of the blood was analyzed in the Van Slyke constant-pressure apparatus according to the method for obtaining the carbon dioxid and oxygen in 1 cc. of blood (4).

The well-known effect of oxygenation of the blood on its carbon dioxid carrying power and the converse effect of carbon dioxid on the amount of oxygen held, will prevent our values from being strictly comparable with the customary standards which have been derived with each of the gases separately. Our chief interest, however, was to obtain results of relative accuracy; and it can hardly be doubted that this was done, within the customary limits of error, in the case of the oxygen capacity of the blood; for the oxygen content of the gas used in the equilibration was not only reasonably constant, but was far beyond the point where slight variations in it would have any determinable effect on the amount of oxygen taken up by the blood.

On the other hand, the success with which a relative accuracy was

obtained in case of the carbon dioxid is less certain. The gas for equilibration of the blood was only occasionally analysed for its carbon dioxid content and it can only be supposed that it was as uniformly constant in this respect as it proved to be on these analyses. This supposition, however, gains much probability from the fact that the gas was always prepared by the same person with as rigid adherence to the technique that has been described as was possible. It hardly seems probable, therefore, that there was any considerable variation in the carbon dioxid content of it.

Collection of the alveolar air: Samples of alveolar air were obtained while the subject was still reclining, before getting up from the metabolism determination; i.e., they are representative of the basal condition. The collection was made by the Haldane-Priestley method (5); inspiratory and expiratory samples were obtained in every case; these were collected over mercury and analysed within a few hours, never later than the same day, in the Haldane analysers that have been described in our first paper (1).

These alveolar samples were obtained in conjunction with practically every metabolism determination, i.e., approximately once a week throughout 1925 and on an average of twice a week during 1926.

Results: 1. *Statistics.* The range of variation shown by these functions is given by the data of table 1.

In the case of the alveolar carbon dioxid percentage we have given the figures for the inspiratory and expiratory samples, separately; for this function we give in addition, and for the other components of the alveolar air we give only the average values.

The inspiratory and expiratory figures are interesting chiefly on account of the difference in variability of the two functions which is indicated by them. Thus for every subject the standard deviation and coefficient of variation are greater for the inspiratory than for the expiratory samples. The meaning of this must be that it is more difficult to deliver a proper inspiratory sample than an accurate expiratory sample; a larger experimental error therefore attaches to the former than to the latter.

The figures for the maxima and minima of the inspiratory and expiratory samples indicate in the cases of C. D., G. H., and K. L. an embarrassment which was common to all of the subjects and from which it was impossible to escape in spite of the most persistent and conscientious efforts; viz., it would occasionally happen that the "inspiratory" sample would contain a higher percentage of carbon dioxid than the "expiratory" sample; with, sometimes but not always, a corresponding inversion of the oxygen values; or, more rarely the oxygen values would be reversed without affecting the carbon dioxid values, at least in sign.

As was just said, none of the subjects were free of this defect; with A. B. it occurred ten times in the total of 116 observations; with E. F., five in fifty; with G. H., six in forty-five; with K. L., five in seventy-eight; but C. D. was the worst offender of all, with a record of fifty-five out of his

TABLE 1

Statistical constants

FUNCTION	SUBJECT	NUMBER OF OBSERVATIONS	MAXIMUM AND MINIMUM	MODE	ARITHMETICAL MEAN	STANDARD DEVIATION	COEFFICIENT OF VARIATION
Alveolar carbon dioxide per cent:							
Inspiratory sample	A. B.	116	4.5–6.2	5.4	5.43 ±0.02	0.300	5.52
	C. D.	129	4.1–6.8	5.7; 5.9	5.75 ±0.02	0.394	6.85
	E. F.	50	5.0–6.2	5.7	5.66 ±0.03	0.308	5.44
	G. H.	45	4.1–5.8	5.0	4.82 ±0.03	0.318	6.61
	K. L.	78	3.5–6.2	5.0	4.87 ±0.03	0.354	7.26
	Average........................				5.31	0.335	6.34
Expiratory sample	A. B.	116	5.3–6.6	5.7	5.81 ±0.02	0.261	4.49
	C. D.	129	4.3–6.5	5.8	5.82 ±0.02	0.389	6.68
	E. F.	50	5.3–6.4	5.9	5.77 ±0.03	0.268	4.64
	G. H.	45	3.9–5.7	5.1	4.97 ±0.03	0.314	6.32
	K. L.	78	3.9–5.9	5.2	5.10 ±0.02	0.292	5.72
	Average........................				5.45	0.305	5.57
Average of the inspiratory and expiratory samples	A. B.	116	5.1–6.2	5.7	5.63 ±0.01	0.215	3.82
	C. D.	129	4.5–6.4	5.8	5.77 ±0.02	0.344	5.96
	E. F.	50	5.2–6.3	5.6	5.72 ±0.03	0.266	4.66
	G. H.	45	4.3–5.6	4.8; 5.0	4.90 ±0.03	0.283	5.77
	K. L.	78	4.0–5.8	5.1	4.98 ±0.02	0.264	5.30
	Average........................				5.40	0.275	5.10
Alveolar carbon dioxid tension	A. B.	116	35.5–43.5	39.5	39.5 ±0.09	1.46	3.70
	C. D.	129	31.5–44.5	41.5	40.6 ±0.14	2.29	5.64
	E. F.	50	36.0–44.0	39.5	40.1 ±0.20	2.08	5.19
	G. H.	45	29.5–39.0	35.0	34.4 ±0.20	2.09	6.07
	K. L.	78	28.5–40.5	35.0	35.0 ±0.20	1.96	5.60
	Average........................				37.9	1.98	5.22
Alveolar oxygen per cent	A. B.	116	13.2–15.6	14.7	14.70 ±0.01	0.494	3.36
	C. D.	129	13.4–16.6	14.6; 15.1	14.78 ±0.03	0.522	3.52
	E. F.	50	13.2–15.7	13.7	14.20 ±0.05	0.576	4.06
	G. H.	45	14.9–16.7	15.7	15.74 ±0.04	0.387	2.47
	K. L.	78	14.4–16.4	15.7	15.67 ±0.03	0.432	2.75
	Average........................				15.02	0.482	3.25
Alveolar oxygen tension	A. B.	116	93–110	105	103.0 ±0.2	3.42	3.32
	C. D.	129	92–118	102	104.0 ±0.3	4.19	4.02
	E. F.	50	92–111	98	99.7 ±0.4	4.27	4.28
	G. H.	45	105–117	111	110.5 ±0.3	2.78	2.51
	K. L.	78	98–123	111	110.0 ±0.3	3.65	3.32
	Average........................				105.4	3.66	3.49

TABLE 1—*Concluded*

FUNCTION	SUBJECT	NUMBER OF OBSERVATIONS	MAXIMUM AND MINIMUM	MODE	ARITHMETICAL MEAN	STANDARD DEVIATION	COEFFICIENT OF VARIATION
Blood carbon dioxid capacity	A. B.	91	49–68	59	58.2 ±0.3	4.37	7.51
	C. D.	88	50–69	57; 58	59.0 ±0.3	4.50	7.62
	E. F.	47	49–63	51	54.5 ±0.4	3.73	6.84
	G. H.	43	45–58	52	52.4 ±0.3	2.86	5.46
	Average				56.0	3.86	6.86
Blood oxygen capacity	A. B.	86	15–25	21	20.8 ±0.1	1.90	9.15
	C. D.	86	17–26	21	21.1 ±0.1	1.73	8.19
	E. F.	44	15–22	19	19.0 ±0.2	1.78	9.39
	G. H.	43	12–18	14	15.7 ±0.2	1.74	11.10
	Average				19.2	1.79	9.46
Barometer	A. B.	116	733–763	753	749	5.41	0.72
	C. D.	129	726–761	746; 750	749	6.40	0.86
	E. F.	50	727–761	748	747	6.08	0.81
	G. H.	45	737–758	749; 752	748	5.16	0.69
	K. L.	78	730–761	751	748	5.98	0.80
	Average				748	5.81	0.78

total of 129. All such instances are of course suspect and perhaps should have been excluded altogether; we have retained those, however, in which the average was well within the normal range, because even if the true inspiratory and expiratory values have not been determined, we had, at least, two carefully obtained samples of the alveolar air whose average, it seemed, could hardly be far from the value being sought.

Even if this is wrong in principle the only subject whose record would be appreciably affected is C. D.; and there is no indirect evidence of anomaly in connection with it except, of course, in the actual distribution and constants of the "inspiratory" and "expiratory" samples themselves as given in table 1. These would be affected for all of the subjects in proportion as they were liable to this error; i.e., as they stand, the data for A. B. and K. L. probably represent more accurately than the others the real differences to be expected between the inspiratory and expiratory values; and these are the ones who show the greatest differences in the mean values of the two samples and in the measures of dispersion and variability.

In spite of the fact that one of the women, E. F., resembes the men more than she does the other two members of her own sex, there is such a striking difference in the mean values of all of these functions for the two sexes that it deserves notice. Thus, for the men, A. B. and C. D., the average alveolar carbon dioxid per cent, 5.70, and tension, 40.1, and the blood-gas

capacity, both for carbon dioxid, 58.6, and oxygen, 21.0, are greater than the corresponding values, 5.20 and 36.5, and 53.4 and 17.4, for the women. On the other hand, the alveolar oxygen per cent, 14.7, and tension, 103.5, for the men are lower than the corresponding values, 15.2 and 106.7, for the women. It is not possible to generalize on the basis of such limited material; the most that can be expected from such a summary is an indication of possibly fruitful lines of attack in future studies of these functions.

With respect to the magnitude of the intra-individual variability as measured by the coefficients of variation, there is no marked average sex difference; it is less in the men for the alveolar carbon dioxid per cent, 4.89, and tension, 4.67, and the blood oxygen capacity, 8.67, than in the women, for whom the corresponding average values are 5.24, 5.42 and 10.24, respectively. By contrast, however, the coefficients of variability average greater for the men for the alveolar oxygen per cent, 3.44, and tension, 3.67, and for the blood carbon dioxid capacity, 7.57, than they do in the women, for whom the corresponding averages are 3.09, 3.37 and 6.15. This lack of a definite or marked sex difference in the measure of gross variability is in spite of the fact that these functions are more indubitably affected by menstruation than any others that we have studied, not excepting the protein and total oxygen consumption (1).

It is difficult to appraise the relative variability of these functions. The preëminent constancy of the alveolar carbon dioxid pressure is too well attested to suffer suspicion from such evidence as this; yet the average coefficient of variability of the carbon dioxid pressure, 5.22, is greater than that of the carbon dioxid percentage, 5.10, or the oxygen percentage, 3.25, or pressure, 3.49. In absolute measure, also, the standard deviation of the carbon dioxid pressure, 1.98, is greater than that of its percentage, 0.275, or of the oxygen percentage, 0.482; only the much higher standard deviation of the oxygen pressure, 3.66, attests to a more rigid regulation of the carbon dioxid tension.

That the effort is made to hold the alveolar carbon dioxid pressure constant even against the slight variations of barometric pressure encountered in this work (see the last item of table 1), is indicated by the following coefficients of correlation:

SUBJECT	COEFFICIENT OF CORRELATION BETWEEN THE BAROMETRIC PRESSURE AND			
	Alveolar carbon dioxid		Alveolar oxygen	
	Per cent	Tension	Per cent	Tension
A. B.	−0.112 ±0.062	+0.090 ±0.063	+0.058 ±0.063	+0.334 ±0.056
C. D.	−0.217 ±0.060	−0.079 ±0.059	+0.316 ±0.053	+0.500 ±0.045
E. F.	0.000	+0.188 ±0.092	−0.143 ±0.094	+0.094 ±0.095
G. H.	0.000	+0.063 ±0.101	0.000	+0.237 ±0.095
K. L.	−0.184 ±0.074	−0.057 ±0.076	+0.245 ±0.072	+0.509 ±0.057
Average	−0.102	+0.042	+0.095	+0.335

These figures show that on the average there is less correlation between the carbon dioxid pressure and barometric pressure than there is between the latter and any of the other variables. Also, the average correlation between the barometric pressure and the carbon dioxid percentage is negative, i.e., the two vary inversely; and the success of this in keeping the carbon dioxid pressure constant is shown by the exceedingly insignificant correlation between the latter and the barometric pressure, 0.042, a figure which is less than the probable error of any of the constituents on which this average is based.

Also, the increased ventilation required to lower the carbon dioxid percentage as the barometer rises will raise the oxygen percentage, and vice versa, so that the oxygen percentage and, even more so, the oxygen tension show a positive correlation, i.e., vary directly as the barometric pressure.

Therefore, in spite of the fact that it was not possible to demonstrate any markedly greater constancy of the carbon dioxid pressure than of the other variables in terms of the measures of variability, the evidence as a whole does show that within the limits of this variation the carbon dioxid pressure is held more constant than the other factors against the fluctuations of the barometer. This will be an important conclusion to bear in mind when we come to consider the possibility of a seasonal variation in these functions.

And finally, a further examination of these correlation constants will show that there is less correlation with the barometric pressure on the part of the women than with the men; in three instances the women show no correlation at all, and in all cases the average for them is less than that of the men. This may be taken to indicate that in the women these functions are subject to some other influence than that of the barometric pressure; a conclusion which will receive confirmation when we come to consider the effects of menstruation.

The blood carbon dioxid capacity is less constant than the oxygen capacity; for, although the average coefficient of variation of the former, 6.86, is less than that of the latter, 9.46, the standard deviations differ in a much greater ratio, being 3.86 and 1.79, respectively. In other words the hemoglobin of the blood is more constant than the alkali reserve.

There is no significant correlation for the group as a whole, between the carbon dioxid tension of the alveolar air and the carbon dioxid capacity of the blood, as is shown by the following tabulation:

Coefficient of correlation between the blood carbon dioxid capacity and the alveolar carbon dioxid tension

A. B.	−0.384 ±0.065
C. D.	−0.301 ±0.067
E. F.	+0.507 ±0.073
G. H.	+0.407 ±0.087
Average	+0.057

It will be noticed that there is a very marked difference in this respect between the two sexes; this will be referred to again in the following section.

II. THE EFFECT OF MENSTRUATION. The effect of menstruation upon these functions and especially the alveolar carbon dioxid percentage and tension is so sharp and clear that it is difficult to see how it has escaped notice up to this time; but if it has been reported we have been unable to find record of it.

Menstruation probably affects many functions of the body; and in our preceding reports (1) (2) we have presented such evidence as our results seem to indicate to be the effects upon metabolism and the cardio-vascular functions. In some cases these confirm and in others contradict previous reports in the literature; and in our own data the effects have been variable; they may show up clearly on the daily graphs for two or three periods and then become confused for several weeks, to reappear and disappear again and again throughout the year. In other words, it is possible to gain assurance of such variations only by averaging large numbers of periods and then one has only an average effect for his pains.

This is mentioned in order to emphasize by way of contrast the effect of menstruation upon the alveolar carbon dioxid percentage and tension. Here it is not necessary to deal with large averages in order to demonstrate an effect; this is so clean-cut and precise that during the something over three years that the three women of this group were under observation there was not a single mentrual period that could not be placed with precision merely by reference to the alveolar carbon dioxid charts. As far as we know this is something that can be said of no other menstrual effect and it is so important and striking that it deserves a special illustration. Figure 1 shows the alveolar carbon dioxid percentage and tension, with the accompanying barometric pressures, for the four subjects (A. B. and C. D., men, E. F. and G. H., women) under observation from June to November, 1925. The gap in the middle of the figure represents the August vacation, and this period was chosen purposely for a reason to be explained later. The two men and the barometric pressures are included by way of controls, in order to make it clear that the effects shown by the women are not due to chance or to simultaneously similar variations in the barometric pressure. The duration of menstruation is shown for each of the women by the heavy short lines underneath their respective graphs. From this figure and also from the average curve of figure 2 it is evident that the carbon dioxid content and pressure in the alveolar air changes in a very definite way during each menstrual cycle; it is lowest just preceding the onset of menstruation and rises to a maximum which is reached in the second or third week following.

The average figures for each of the subjects for this as well as the other functions reported here are given in table 2; and the grand averages are

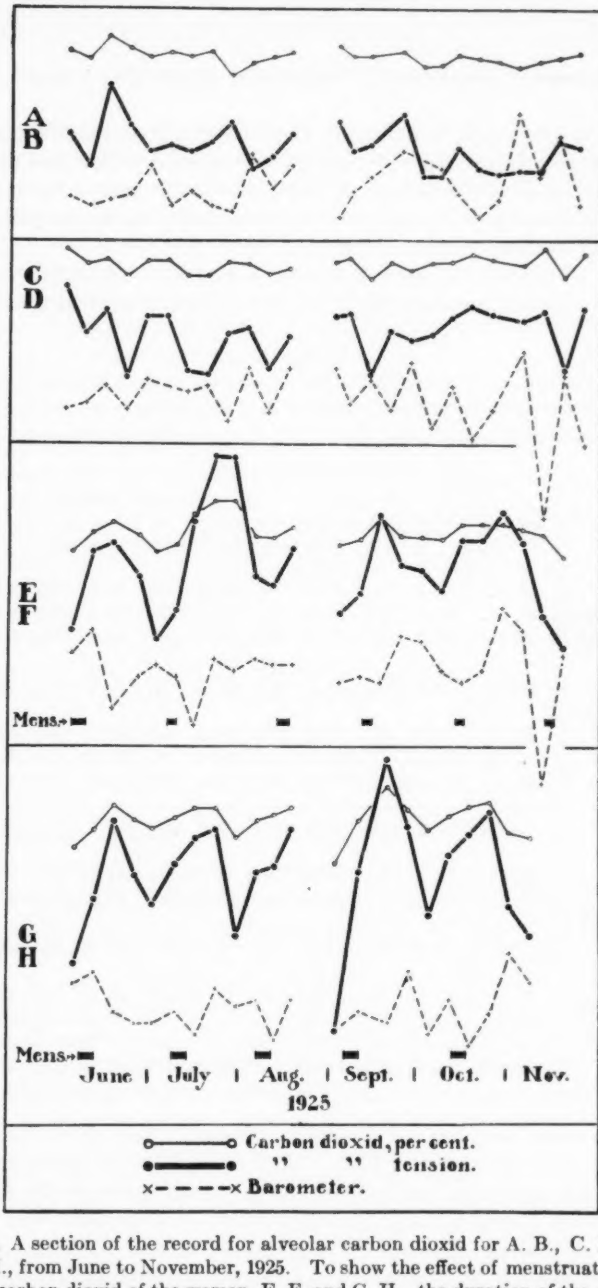


Fig. 1. A section of the record for alveolar carbon dioxid for A. B., C. D., E. F., and G. H., from June to November, 1925. To show the effect of menstruation on the alveolar carbon dioxid of the women, E. F. and G. H., the duration of the menstrual periods is indicated by the short, heavy lines beneath these curves. The two men, A. B. and C. D. and the curves for the barometric pressures are included by way of "controls" (see text).

represented graphically in figure 2. From these it is seen that the alveolar oxygen tension varies in a way that is the reciprocal of that just described for the carbon dioxid; it is highest during the latter part of the intermenstrual period and falls during menstruation itself to its lowest point in the first half of the intermenstrual period.

It may be again mentioned, here, that one of the women (K. L., 1926) furnished no blood for analysis; the evidence for a menstrual effect on the

TABLE 2

The effect of menstruation. Averages of the observations made on the women during the menstrual periods and during each week of the inter-menstrual period

The numbers in parentheses are the number of observations on which each average is based.

FUNCTION	SUBJECT	MENSTRUAL PERIOD	INTERMENSTRUAL PERIOD			
			First week	Second week	Third week	Fourth week and longer
Alveolar carbon dioxid tension	E. F.	39.7 (6)	40.7 (14)	41.2 (9)	39.6 (10)	39.1 (11)
	G. H.	34.3 (7)	35.3 (10)	36.0 (9)	33.5 (9)	32.6 (9)
	K. L.	34.5 (6)	35.9 (19)	35.9 (19)	34.4 (16)	33.3 (17)
	Average	36.1 (19)	37.3 (43)	37.2 (37)	35.6 (35)	38.4 (37)
Alveolar oxygen tension	E. F.	99.4 (6)	99.2 (14)	99.2 (9)	100.5 (10)	99.7 (11)
	G. H.	110.8 (7)	109.1 (9)	109.8 (8)	111.2 (9)	111.8 (8)
	K. L.	111.0 (6)	109.9 (19)	109.4 (19)	110.2 (16)	110.3 (17)
	Average	107.3 (19)	106.2 (42)	107.0 (36)	107.7 (35)	107.4 (36)
Blood carbon dioxid capacity	E. F.	52.32 (5)	56.27 (14)	54.68 (9)	54.13 (9)	53.49 (10)
	G. H.	50.86 (7)	52.53 (10)	53.29 (9)	52.36 (10)	52.48 (7)
	Average	51.47 (12)	54.71 (24)	53.99 (18)	53.20 (19)	53.07 (17)
Blood oxygen capacity	E. F.	18.39 (5)	18.96 (13)	19.13 (8)	19.19 (8)	19.06 (10)
	G. H.	15.69 (7)	16.29 (10)	15.10 (9)	16.11 (10)	15.85 (7)
	Average	16.82 (12)	17.80 (23)	17.00 (17)	17.48 (18)	17.74 (17)

blood gas capacity is derived, therefore, from only the two women, E. F. and G. H., who served during 1925. Their data, however (table 2 and fig. 2), are quite unanimous with respect to the blood carbon dioxid capacity; this is lowest during menstruation and reaches its highest point in the first or second week following. The similarity of this to the alveolar carbon dioxid curve is striking; and a relationship between the two is further suggested by the coefficients of correlation which were given at the end of the preceding section. The average coefficient of correlation between the

alveolar carbon dioxid pressure and the blood carbon dioxid capacity was +0.457 for the two women; and the significance of this, in itself rather high figure, is enhanced by the fact that each of the men showed a negative correlation, average −0.342, so that the total average correlation for the group as a whole was insignificant.

The blood oxygen capacity is the only function of this group which suffers from any marked dissimilarity in the different subjects. The course of the two curves (table 2) agrees very well with the exception of an apparently abnormally low value for G. H., in the second inter-menstrual week. The average curve (fig. 2) appears quite irregular on account of this single and possibly erratic value; excluding this, the blood oxygen capacity is lowest for both subjects during menstruation itself; and this is true of the average result even if this value is retained.

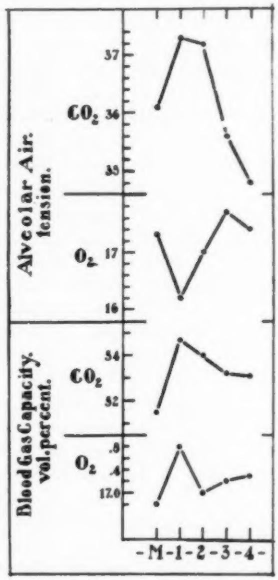


Fig. 2. Average effect of menstruation on the composition of the alveolar air and the blood gas capacity. *M*, *1*, *2*, *3*, and *4* are respectively, the menstrual period and the first, second, etc., weeks of the inter-menstrual period.

III. Seasonal variation. Our evidence on this subject is given by way of monthly averages in table 3 and the grand averages calculated therefrom are shown graphically in figure 3.

1. The alveolar air. The observations which we have made on the alveolar air throughout the two years of this study are more difficult to appraise with respect to a possible seasonal periodicity than those which we have for any other function. Had we nothing but the data for either of the two years separately it would be very easy to believe in a definite seasonal variation. To be sure, in each year there is the same lack of exact, inter-individual concurrence with respect to the incidence of the maximum and minimum values which these subjects have shown in other functions. But on the whole there is sufficient agreement to give average curves (fig. 3), especially for carbon dioxid, which are unusually smooth and convincing. The great difficulty in the interpretation of these data, however, and the one before which all minor discrepancies become relatively unimportant is this: whereas the carbon dioxid percentage and tension are highest during the summer of 1925 they completely reverse themselves and are lowest during the summer of 1926; and the converse is true, with some slight irregularities, for the alveolar oxygen.

TABLE 3

Monthly averages for the blood gas capacities and the alveolar air

SUBJECT	DATE	BAROMETER	TEMPERATURE: Outdoor, 8 a.m.	TEMPERATURE: Room	ALVEOLAR AIR: Carbon dioxid: Per cent	ALVEOLAR AIR: Carbon dioxid: Tension	ALVEOLAR AIR: Oxygen: Per cent	ALVEOLAR AIR: Oxygen: Tension	ALVEOLAR AIR: Number of observations	BLOOD-GAS CAPACITY: Volume per cent: Carbon dioxid	BLOOD-GAS CAPACITY: Volume per cent: Oxygen	BLOOD-GAS CAPACITY: Number of observations
			°C.									
E. F.	1925 Feb.	742.0	−1.1	25.0	5.50	38.3	14.73	102.0	4	51.95	17.42	4
	Mar.	745.0	5.0	24.0	5.55	38.8	14.83	103.0	4	51.77	16.24	4
	Apr.	748.0	5.6	24.0	5.48	38.5	14.48	102.0	5	53.03	17.24	5
	May	746.0	7.2	25.0	5.73	40.2	14.62	103.0	3	55.95	18.39	3
	June	748.8	16.1	24.7	5.68	39.9	14.22	100.0	4	53.00	19.60	4
	July	746.5	18.9	24.2	5.89	41.3	13.79	96.7	5	52.37	18.37	5
	Aug.	749.4	20.0	25.1	5.71	40.1	13.73	96.6	3	51.33	18.73	3
	Sept.	748.4	16.7	22.5	5.70	40.1	13.98	98.2	4	54.55	18.96	4
	Oct.	750.9	6.1	23.0	5.79	40.8	13.89	97.9	5	57.62	20.71	4
	Nov.	744.5	−0.5	25.6	5.58	39.0	14.64	102.4	3	57.11	20.75	3
	Dec.	746.0	−2.8	23.5	5.84	40.9	14.12	98.9	4	58.58	21.49	4
	1926 Jan.	750.1	−10.6	23.5	5.97	42.0	13.75	96.7	4	58.77	20.45	2
	Feb.	751.2	−8.3	24.0	5.88	41.4	13.72	96.8	2	58.06	19.78	2
G. H.	1925 Feb.	748.0	−15.0	23.0	4.58	32.1			1	51.53	16.92	2
	Mar.	753.0	2.8	25.0	4.63	32.7	16.14	114.0	4	51.49	14.87	4
	Apr.	749.0	5.6	25.0	4.79	33.2	16.07	113.0	4	49.01	14.91	4
	May	748.0	7.8	25.0	4.85	34.1	15.67	110.0	5	52.83	15.81	5
	June	749.4	17.8	24.5	4.92	34.6	15.64	110.1	4	53.10	15.63	4
	July	746.7	17.2	23.5	5.01	35.1	15.67	109.9	5	52.39	16.67	5
	Aug.	746.8	20.6	25.1	5.10	35.8	15.58	109.1	3	50.18	16.93	3
	Sept.	747.6	18.3	22.8	5.01	35.2	15.55	109.3	4	51.71	16.18	4
	Oct.	744.7	6.1	23.1	5.12	35.8	15.44	107.8	4	54.47	16.56	3
	Nov.	754.3	−0.5	25.1	4.73	33.5	15.90	112.7	2	54.15	16.79	2
	Dec.	748.9	−5.6	23.9	4.90	34.4	15.77	110.9	4	54.30	15.41	3
	1926 Jan.	743.1	−3.9	23.9	4.88	34.0	15.56	108.3	3	53.99	14.34	3
	Feb.	746.3	−8.9	23.2	4.72	33.1	15.85	111.0	2	54.71	14.93	1
K. L.	1926 Feb.	744.4	−3.3	23.8	5.07	35.4	15.18	106.1	3			
	Mar.	745.5	−5.0	24.2	4.91	34.3	15.58	108.9	9			
	Apr.	748.8	−1.1	24.6	4.96	34.9	15.49	108.8	9			
	May	748.9	5.6	24.1	5.04	35.8	15.85	111.4	8			
	June	746.3	14.4	24.4	4.72	33.3	15.97	112.6	8			
	July	750.7	18.9	24.5	4.92	34.6	15.89	111.8	7			
	Aug.	751.1	18.3	25.5	4.86	34.2	15.66	110.2	5			
	Sept.	751.9	13.9	24.5	5.12	36.0	15.51	109.4	8			
	Oct.	743.7	7.8	25.5	5.11	35.6	15.52	108.1	6			
	Nov.	749.3	5.6	27.0	5.05	35.5	15.69	110.2	6			
	Dec.	749.9	−2.2	25.7	4.93	34.7	15.81	111.2	7			
	1927 Jan.	743.8		25.4	5.35	37.3	15.12	105.4	3			

TABLE 3—*Concluded*

SUBJECT	DATE	BAROMETER	TEMPERATURE: Outdoor, 8 a.m.	TEMPERATURE: Room	ALVEOLAR AIR: Carbon dioxid: Per cent	ALVEOLAR AIR: Carbon dioxid: Tension	ALVEOLAR AIR: Oxygen: Per cent	ALVEOLAR AIR: Oxygen: Tension	ALVEOLAR AIR: Number of observations	BLOOD-GAS CAPACITY: Volume per cent: Carbon dioxid	BLOOD-GAS CAPACITY: Volume per cent: Oxygen	BLOOD-GAS CAPACITY: Number of observations
			°C.									
A. B.	1925 Feb.	751.0	0.5	26.5	5.57	39.3	14.46	102.0	2	51.85	17.43	3
	Mar.	750.0	0.5	23.0	5.72	40.3	14.52	102.0	4	54.90	16.64	4
	Apr.	753.0	6.7	26.0	5.92	41.9	13.84	98.0	4	52.20	18.40	3
	May	750.0	6.7	24.0	5.83	41.0	14.31	101.0	4	53.71	20.03	4
	June	748.5	15.0	24.6	5.80	40.8	14.64	102.8	5	55.87	20.02	5
	July	746.3	20.0	25.5	5.78	40.5	14.59	102.2	4	50.46	20.02	4
	Aug.	752.0	20.0	24.6	5.67	40.1	14.12	100.1	3	52.13	19.61	3
	Sept.	749.8	15.6	23.0	5.77	40.7	14.66	103.5	4	55.27	20.57	4
	Oct.	749.0	7.2	23.9	5.61	39.5	14.91	104.9	5	57.05	20.39	5
	Nov.	755.0	1.1	24.8	5.60	39.7	15.12	107.2	5	57.39	20.37	5
	Dec.	746.5	−0.5	23.3	5.59	39.2	15.00	105.1	3	59.86	23.07	3
	1926 Jan.	748.2	−1.7	24.1	5.63	39.6	15.12	106.0	4	58.08	20.48	4
	Feb.	746.5	−5.6	23.6	5.66	39.6	14.65	102.6	5	61.56	22.12	4
	Mar.	747.6	−3.3	24.0	5.54	38.9	14.72	103.2	8	59.50	20.71	5
	Apr.	747.1	1.7	25.2	5.45	38.2	14.85	104.1	6	58.95	21.94	2
	May	745.4	7.2	25.1	5.50	38.5	14.72	102.7	8	60.07	20.92	3
	June	746.5	14.4	24.7	5.73	40.2	14.38	100.6	9	59.72	20.70	4
	July	749.2	16.7	24.7	5.64	39.6	14.70	103.1	6	57.79	20.35	3
	Aug.	750.4	20.0	26.1	5.48	38.6	14.92	101.9	4	53.82	19.84	2
	Sept.	751.1	14.4	24.7	5.40	38.1	15.14	106.7	8	61.66	21.77	4
	Oct.	744.9	9.4	25.4	5.58	39.0	14.64	101.6	4	63.70	22.91	4
	Nov.	749.9	5.6	26.6	5.50	38.7	14.79	103.4	5	62.22	23.19	5
	Dec.	749.8	−1.1	26.1	5.50	38.7	14.93	105.1	4	63.34	22.57	3
	1927 Jan.	747.3		25.2	5.74	40.3	14.30	100.5	2	65.19	22.72	2
C. D.	1925 Feb.	750.0	4.4	26.3	5.82	40.9	15.26	108.0	2	51.85		2
	Mar.	754.0	−3.9	22.0	5.74	40.7	15.39	109.0	5	58.37	18.33	5
	Apr.	754.0	2.2	25.0	5.90	41.8	14.78	107.0	4	53.65	18.35	3
	May	748.0	5.0	23.0	6.18	43.4	14.50	102.0	4	61.53	19.88	4
	June	748.0	17.8	24.7	6.13	43.1	14.37	101.0	5	54.57	19.75	5
	July	748.3	21.1	24.7	6.02	42.3	14.77	103.8	4	52.39	19.35	4
	Aug.	750.6	21.1	24.0	6.02	42.8	14.69	103.6	3	53.26	20.72	3
	Sept.	750.1	16.7	22.9	6.07	42.8	14.56	102.6	5	56.33	20.77	5
	Oct.	744.8	6.1	24.3	6.20	43.4	14.20	99.3	4	58.50	20.96	4
	Nov.	743.6	4.4	25.1	6.17	43.1	14.39	100.6	4	58.26	21.93	4
	Dec.	744.8	−2.2	24.9	6.16	43.1	14.23	99.6	4	58.84	20.17	4
	1926 Jan.	746.3	−9.4	24.5	5.78	40.5	14.49	101.5	4	56.91	20.90	4
	Feb.	744.9	−6.1	23.3	5.85	41.0	14.73	103.1	5	55.81	22.08	4
	Mar.	745.1	−4.4	23.8	5.87	41.1	14.68	102.8	8	59.86	21.38	5
	Apr.	749.1	−1.7	23.8	5.70	40.2	14.79	104.6	8	62.90	21.09	3
	May	749.1	5.6	23.8	5.79	40.8	14.70	103.3	8	61.54	21.94	4
	June	746.2	13.3	23.9	5.53	39.1	14.85	105.1	8	61.06	21.93	5
	July	748.7	18.9	24.8	5.33	37.5	15.33	107.9	9	58.84	20.06	2
	Aug.	751.2	18.3	25.6	5.57	39.4	14.69	103.6	5	57.08	20.76	3
	Sept.	753.2	12.2	24.4	5.57	39.3	14.82	106.4	7	67.99	23.24	2
	Oct.	748.2	7.8	25.4	5.56	39.2	14.55	102.1	8	65.06	22.72	4
	Nov.	747.2	5.0	26.0	5.63	39.5	14.94	104.6	6	65.84	23.77	4
	Dec.	754.4	−6.1	25.4	5.51	39.0	15.16	107.5	6	65.35	23.08	3
	1927 Jan.	744.4		25.0	5.76	40.2	14.67	102.6	3	65.38	23.92	2

Our result for 1926 has the advantage of support from previous work; for Boycott and Haldane (6) and Lindhard (7) have reported a lowering of the alveolar carbon dioxid tension during the warmer parts of the year.

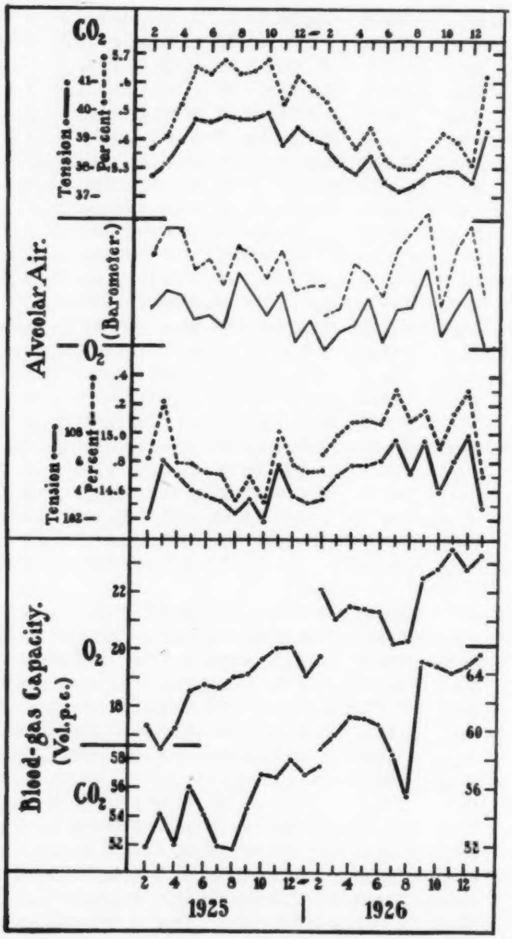


Fig. 3. Seasonal variation in alveolar air (upper set of curves) and blood gas capacity (the two lower curves). Each curve, except the continuous line for the barometric pressure, represents the monthly grand averages calculated from the individual averages of table 3. The continuous line for the barometric pressure represents the monthly averages supplied by the local Weather Bureau office. Both the alveolar carbon dioxid and oxygen are shown for percentage (dotted lines) and tension (continuous lines).

But until it can be shown that our 1925 result was entirely fortuitous and due to accidental causes of which we are at present not only unaware but entirely without suspicion this can only mean that whatever factors influence the composition of the alveolar air they are such as to *usually* produce a lowering of its carbon dioxid tension during the summer. This provides a criterion by which to test the validity of those explanations that have previously been advanced to account for this phenomenon.

Boycott and Haldane found that transition from a cold room (5–10°) to the laboratory (15–18°) and to a hot room (25–35°) in any sequence caused a change in the composition of the alveolar air in the sense that "exposure to cold causes a slight rise and to heat a slight fall in the alveolar carbon dioxid pressure." From a comparison of observations made during the spring, summer and fall they concluded, in addition, that this "influence of temperature also seemed to be effective in producing a general seasonal variation in the average normal alveolar carbon dioxid tension determined at room temperature. . . . . The temperature of the air of the laboratory on all the occasions mentioned lay between 14° and 19°." In this connection it is not clear whether they attributed the seasonal effect to the outdoor temperature or to such seasonal variation of the laboratory temperature as might have occurred within the limits 14–19°. Although they give no figures to show that variation of the environmental temperature within these limits is capable of affecting the alveolar carbon dioxid tension, this seems to be implied from their further statement that this temperature effect "has nothing to do with body temperature as measured in the rectum, and is produced by contact of warm air with the face and hands *within two or three minutes*" (italics ours).

That the outdoor temperature cannot be the controlling factor is suggested also by our data for the two years covered by this investigation. In table 3 are given the average monthly outdoor temperatures that our subjects were exposed to on their way to the laboratory on the mornings of the tests; these are compiled from the Weather Bureau readings for 8 a.m. It can easily be seen that the outdoor temperature varied quite uniformly and identically during each of the two years and cannot therefore provide an explanation for the reversal of the carbon dioxid variation which we have observed during these same periods.

But the alveolar air samples were not taken until after the completion of the basal metabolism determinations, which with the preliminary rest period of forty-five to sixty minutes necessitated an exposure of one hour and a half to two hours to the temperature of the laboratory before sampling the alveolar air. If then, as Boycott and Haldane claim, the adjustment of the alveolar carbon dioxid pressure to a change in temperature occurs within two or three minutes it remains to be seen whether variations in the temperature of the laboratory air may account for the observed changes in the alveolar carbon dioxid.

The average, monthly temperatures to which each subject was exposed during the detprminations are also given in table 3. It can be seen that they, also, fail to show any correlation with the variations in alveolar carbon dioxid tension. And further, individual determinaions show that the effect described by Boycott and Haldane does not operate within the relatively narrow variation of laboratory temperature encountered in this (as well as their own) work; nor is the alveolar carbon dioxid pressure constant for similar laboratory temperatures and barometric pressures. The latter point may be shown first in the following tabulation of data taken at random from the records of A. B. but quite typical of all of the subjects:

BAROMETER	ROOM TEMPERATURE	ALVEOLAR CARBON DIOXIDE PRESSURE
753	23.0	41.2 (May); 38.7 (Mar.)
753	23.5	39.4 (Mar.); 37.4 (Sept.)
752	24.0	40.4 (May); 38.4 (Sept.)
748	24.5	40.7 (June); 39.5 (Mar.)
745	25.0	41.1 (July); 39.7 (Dec.)
752	25.0	41.0 (May); 38.9 (Sept.); 39.3 (Jan.)
751	25.0	39.5 (June); 38.1 (Oct.)
749	25.0	39.9 (Aug.); 37.5 (Sept.)
748	25.0	40.2 (Oct.); 39.2 (May)
745	25.0	41.1 (July); 38.9 (Sept.); 39.7 (Dec.)
752	25.5	38.0 (May); 37.3 (Aug.)
746	25.5	41.6 (June); 43.2 (June); 40.4 (July)
749	26.0	40.1 (Feb.); 43.7 (May); 40.1 (July)
753	26.0	40.0 (Aug.); 39.6 (Oct.); 40.7 (Aug.)
753	27.0	38.4 (Feb); 42.1 (Apr.); 37.3 (Nov.)

The conclusion from this must be that something besides the laboratory temperature must be operating to modify the composition of the alveolar air over the periods of time covered by this work.

BAROMETER	ROOM TEMPERATURES												
	20.5°C.	21.5°C.	22.0 °C.	23.0 °C.	23.5 °C.	24.0 °C.	24.5 °C.	25.0 °C.	25.5 °C.	26.0 °C.	26.5 °C.	27.0 °C.	28.0 °C.
	Alveolar carbon dioxid pressures												
mm.													
757		39.2		38.9	38.9			39.0					
754	39.2	40.1				39.3	36.3		38.4		39.2		
753				39.9	38.4		42.1	42.7	37.5	40.1		39.3	
752			39.6		39.2	39.4		39.7	37.6				
751			39.3		40.3	42.4		38.8			37.2		39.4
750				38.8		40.9	40.9	38.7	38.6	40.1			
749								38.7		41.1			
747						39.6	38.0		37.6	42.4		39.3	
746						39.5	38.2	40.2	41.7	40.0		39.6	
743			39.0			39.3	39.3	39.0	41.2				

The above tabulation, also of data from the records of A. B., bears more specifically on the point raised by Boycott and Haldane; and shows that within the limits of room temperatures encountered in our work it is quite impossible to explain the variations in the composition of the alveolar air according to their rule that "exposure to cold causes a slight rise and to heat a slight fall in the alveolar carbon dioxid pressure:"

These represent all of the observations on A. B. in which any considerable temperature range was covered at any barometric pressure; these combinations occurred by chance throughout the two years covered by this work and they therefore have a particular bearing on the contention of Boycott and Haldane insofar as they attempt to apply it to the explanation of seasonal variations; this is disproved by these figures, it seems to us, rather completely.

Since, therefore, there is no observable relation between the average monthly outdoor or room temperature and alveolar carbon dioxid; since we may have widely different alveolar carbon dioxid pressures at identical combinations of barometer and room temperature at different times of the year; and since we may have nearly identical alveolar carbon dioxid tensions at the different room temperatures encountered in this work (20°–28°); we must conclude that the explanation proposed by Boycott and Haldane to account for seasonal variations in the alveolar carbon dioxid tension is, at least, not applicable to our results.

For reasons similar to but less detailed than these, Lindhard also abandoned the theory of Boycott and Haldane as an explanation of seasonal variations in the composition of the alveolar air and substituted for it one of his own which involved the action of sunlight. As the result of the effects which he and others had found to follow light-baths he concluded "that the annual fluctuation in the alveolar carbon dioxid tension is due to the action of the sunlight through an increase in the excitability of the respiratory center towards carbon dioxid" (7, p. 288). This would satisfactorily explain such results as he obtained and our own results for 1926 when the alveolar carbon dioxid was lowest during the summer. But there is no need to labor the incompatibility of such an hypothesis with our result for 1925. It has not seemed necessary to reproduce here the Weather Bureau statistics for the total hours or percentage of sunshine for this locality for the two years, 1925 and 1926; primarily because such figures would not describe accurately the exposure of each of the subjects individually. Needless to say, however, the available sunshine was in no marked degree dissimilar for the respective seasons of the two years; and as far as it can be known there was no significant, if in fact there was any difference at all in the amount of exposure of the subjects during the two years.

It remains to mention only one final point which bears somewhat more directly on the very basis of Lindhard's theory and therefore upon its

general validity. It has been mentioned, before, that during their vacations, which occurred in both years during the last two weeks of August, both A. B. and C. D. enjoyed vigorous outdoor activity; during 1925 they were both on a ten-day canoe trip; in 1926, this was repeated by C. D. while A. B. spent much time on a Lake Erie beach near Buffalo; and in both cases there was much exposure and tanning. It is interesting, therefore, to compare their alveolar carbon dioxid tensions before and after these "light-baths." This is done graphically for 1925 in figure 1; the average figures for July and the part of August preceding, and for the month of September following the vacations of each year are as follows:

SUBJECT		JULY	AUGUST	VACATION	SEPTEMBER
A. B.	1925	40.5	40.1		40.7
	1926	39.6	38.6		38.1
C. D.	1925	42.3	42.8		42.8
	1926	37.5	39.4		39.3

Only in the case of A. B. for 1926 was the average for September measurably lower but even this does not differ as much from the value for the first part of August as the latter from the July average. On the whole, therefore, and by way of summary, it seems impossible to agree with Lindhard as to the importance of sunlight in causing such changes in the alveolar carbon dioxid tension as we have observed.

Our own opinion is that even within the limits of its normal fluctuations the barometric pressure is the most important, single factor in causing these seasonal variations in the composition of the alveolar air.

This seems quite obviously so in regard to the alveolar oxygen. It has been shown in a previous section that there is a fairly high correlation, +0.5 for two of the subjects, and +0.3 for the group as a whole, between the day-to-day variations of the alveolar oxygen tension and the barometric pressure at the time of the determinations. A parallelism perhaps somewhat better than this seems to be shown by at least four of the subjects (A. B., C. D., G. H., and K. L.) when we consider the monthly averages given in table 3; and when we pass on to the grand averages calculated from these and shown graphically in figure 3 a remarkably close correspondence is seen between the curves for barometric pressure and alveolar oxygen percentage and tension.

This increasing degree of correlation as we get farther away from the basic, daily determinations would seem to involve something more than the mere balancing of errors which is the usual benefit to be derived from the use of averages. This is suggested immediately by a consideration of the data for E. F., who was distinguished by omission from the roll-call in the preced-

ing paragraph. If we refer again to the curve for the total average barometric pressure for 1925, figure 3, it will be seen that there is a fairly uniform decline from a high value in March to the low value for December. This decline is shown, with minor irregularities by the monthly averages of the barometric readings for each of the subjects, individually, with the exception of E. F. Curiously enough, the observations on this subject were made on such atypical days throughout this whole period that the monthly averages of the barometric readings made at the time of the determinations show a progressive rise from February 1925 to February 1926. And yet, the important point is that the curve for her alveolar oxygen conforms very closely to those of the other subjects and to that for the grand average in figure 3. In other words, her alveolar oxygen varied, not as the barometric readings at the time of the determinations but in conformity with the *average* barometric pressure.

This is a matter that will be increasingly important in a moment when we come to a consideration of the carbon dioxid; what it seems to imply is that there is sufficient lag in the adjustment of the composition of the alveolar air to the fluctuations of the barometer to prevent any close correlation with the immediate barometric pressure.

It is only by such an assumption, it seems to us, that it is possible to account for our data regarding the carbon dioxid. If the average curves for the barometric pressure and the alveolar carbon dioxid percentage and tension of figure 3 are examined there seems to be conclusive evidence of a negative correlation; there are minor discrepancies, but on the whole the curves are very accurate reciprocals of each other. This relationship is shown also by the monthly averages of table 3, with, as before, E. F. the most outstanding exception. But, again, in spite of the fact that her barometric pressure curve is quite atypical her carbon dioxid curves are in conformity with those of the other subjects and, like them, inversely related to the *average* barometric pressure.

When we consider the daily determinations we find only a very slight, negative, average correlation, -0.102, between the carbon dioxid percentage and barometric pressure; and that the average correlation is even less than this and curiously enough positive, $+0.042$, between the alveolar carbon dioxid tension and the barometric pressure. This difference, especially in sign, between the coefficients for percentage and tension is particularly interesting when compared to the relation between the two shown in the monthly and grand averages of table and figure 3; as can be seen at a glance at the figure, the two vary *pari passu*. Thus it is worthy of note that by the month or year the carbon doxid tension is not held constant; as the barometer changes, the compensatory alterations in the carbon dioxid percentage are, apparently, carried by their own momentum sufficiently beyond the requirements of a precise adjustment to produce a

variation of the tension in the same sense; therefore, in the long run, the parallelism which we find between the two. But at any instant of sampling the alveolar air, if it is in process of adjustment to a changing barometer, it is not impossible that there may be sufficient lag in the alterations of its percentage to produce for the moment a change in tension of the same sign as the changing barometric pressure.

In conclusion, the composition of the alveolar air seems from our data to be more definitely correlated—the oxygen positively and the carbon dioxid negatively—with the barometric pressure than with any other environmental variable; this is more apparent for the averages and for long periods of time than it is in the individual determinations—in part, no doubt, on account of the inescapable errors of observation; but, even more, we believe as a result of a lag in the adjustment between the two, whereby the correlation becomes more apparent between the average values.

2. Seasonal variation in the blood gas capacity. It will be recalled that our data in regard to the blood gas capacity are derived from only four of the subjects, A. B. and C. D. serving through both years; E. F. and G. H. in 1925; while K. L., 1926, furnished no blood samples. The monthly averages are given in table 3 and the grand averages, calculated thereform, are shown graphically in figure 3.

The results are so uniform and consistent that they require very little comment. The summer depression in the blood carbon dioxid capacity which is so apparent in the average curve of figure 3 is attested by all of the subjects individually and with such precision and concurrence that it may be regarded, together with the basal oxygen consumption and basal pulse rate, as one of the most definite seasonal variations which we have observed.

On the other hand the data for the blood oxygen capacity are in less complete agreement. The data for each year, separately, are quite consistent; thus for all of the subjects the lowest values during 1925 are in the spring; there is, in addition, a very slight depression in the records of A. B., C. D., and E. F. during July and August. In 1926 both subjects, A. B. and C. D., show a very uniform and concurrent lowering in the summer with no suggestion of the spring depression of the previous year. Since, therefore, the summer depression is shown to at least some extent in both years, and since the early spring determinations of 1925 might have suffered from more than average error while the technique was becoming standardized, it seems probable that the summer depression is the more typical and likely result.

We have no explanation to advance for these phenomena; and in conclusion would call attention to only one other aspect of variation shown by the blood gas capacity, viz., the gradual, upward slope of both curves throughout the two years. This is shown by all of the data except the blood oxygen capacity of G. H.; and for A. B. and C. D., who served during

the entire two year period the increases are remarkably alike, as may be seen from the following figures taken from table 3:

		Carbon dioxid	Oxygen
A. B.	Feb., 1925	51.85	17.43
	Feb., 1927	65.19	22.72
	Diff.	13.34	5.29
C. D.	Feb., 1925	51.85	18.33 (March)
	Feb., 1927	65.38	23.92
	Diff.	13.53	5.59

As far as known there was no progressive modification of the technique that could account for such a change; it must be of physiological origin and if so we are unable to explain it, except, perhaps, as the result of the regular withdrawal of the 15–20 cc. blood samples each week. It is possible that this may have served as a stimulus to blood regeneration; but if so, it failed to make any corresponding effect on the blood-cell counts which will be presented in a later paper; nor, as far as we can tell, did it have any effect on any of the other functions. It was largely in order to serve as a control against this possibility that no blood samples were taken from K. L. during 1926.

SUMMARY

This is the third installment of a series (1) (2) describing the physiological changes observed in two men and three women during the two-year period 1925–1927; this paper deals with the composition of the alveolar air and the blood gas capacity.

Statistical measures are given of: the intra-individual variability of these functions; the degree of correlation between the components of the alveolar air and the barometric pressure; and the correlation between the alveolar carbon doxid tension and the blood carbon dioxid capacity.

The most significant datum in this report is the strikingly clean-cut effect of menstruation on the alveolar carbon dioxid percentage and tension; this is the most definite and indubitable effect of menstruation that we have observed in all of the functions for which we have kept record. This effect as well as the effect on the alveolar oxygen and the blood gas capacity is so concisely summarized in table 2 and figure 2 that it need not be repeated here.

The alveolar air shows a seasonal variation in composition which we believe is related, the oxygen directly and the carbon dioxid inversely, to the average barometric pressure.

The blood gas capacity also shows a seasonal variation; the carbon dioxid capacity is quite conclusively lowest during the summer; this is also true of

the oxygen capacity for 1926 and reasons are given for believing that this is probably the typical effect. In addition, the blood gas capacity shows a progressive increase throughout the two years which we are unable to account for except, perhaps, as a stimulating effect on blood regeneration of taking the weekly blood samples.

BIBLIOGRAPHY

(1) Griffith et al. This Journal, 1929, lxxxvii, 602.
(2) Griffith et al. Ibid., 1929, lxxxviii, 295.
(3) Plesch, J. Zeitschr. f. exper. Path. u. Therap., 1909, vi, 380.
(4) Van Slyke, D. D. and W. C. Stadie. Journ. Biol. Chem., 1921, xlix, 35.
(5) Haldane, J. S. and J. G. Priestley. Journ. Physiol., 1905, xxxii, 225.
(6) Boycott, A. E. and J. S. Haldane. Ibid., 1908, xxxvii, 355.
(7) Lindhard, J. Skand. Arch. f. Physiol., 1912, xxvi, 266.

EXPLANATION OF WEDENSKY INHIBITION

PART I

G. KATO, T. HAYASHI, T. OTA, M. NAKAYAMA, H. TAMURA, M. TAKEUCHI, K. KANAI AND S. MATSUYAMA

From the Physiological Institute, Keio University, Tokyo

Received for publication April 6, 1929

Wedensky (1) found that at a certain stage in the fatigue or narcosis of a nerve-muscle preparation, a series of strong or rapidly recurring stimuli, applied to the nerve, may produce a small initial contraction only (Anfangszuckung), whereas a series of weak or slowly recurring stimuli produces a continued tetanus. In a more recent paper (2) he described observations, showing that the same effect may be obtained not only by local narcosis of nerve, but also by local application of strong solutions of electrolytes or a high temperature. This is "paradoxes Stadium" in his sense.

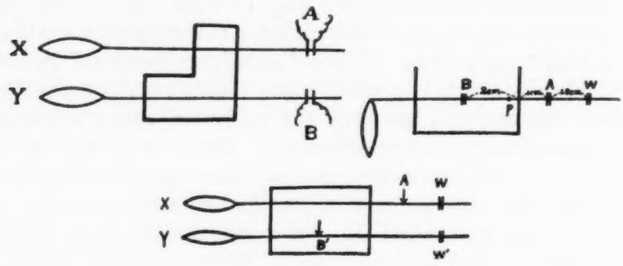
The "Wedensky effect," by which physiologists usually understand this paradoxical phenomenon, has been the subject of many researches and various explanations have been advanced. However there is no need to recall all of them, as they can be found in a paper published by Lucas (3). Lucas reviewed the previous explanations and offered a new one. Adrian (4) supported and completed it. Their explanation is as follows: the narcotized area is a region of decrement. When the rate of stimulation is increased the stimuli may fall in the relative refractory period at the time when the irritability of the nerve at the stimulated point is below normal so that smaller impulses are aroused which can not pass through the region of decrement. An increase in strength of stimuli may have the same effect, since the stronger stimuli would excite the nerve earlier in the period of recovery and thus give rise to the smaller impulses none of which would be large enough to pass through the region of decrement. Thus their explanation is based upon the decremental conduction in narcotized region. In reading their papers, one can not fail to be struck by the critical acumen with which they handled the evidence which was available when they wrote.

However the decremental conduction in a narcotized region was disproved by Kato (5) and Forbes (6), and quite recently by Davis and Rice (7). Furthermore Kato has found the sources of error which had led to the inference of conduction with decrement: 1, a diffusion of narcotic out of the chamber and a diffusion inwards of normal tissue fluid (8); 2, lack of uniformity in the depth of narcosis along the nerve (9); 3, the effect

due to the spread of the stimulating current. Therefore the Wedensky effect is left unsolved as a new question to be dealt with from the standpoint of "decrementless conduction".

In part I we will deal with the point of the narcotized nerve at which the series of subnormal impulses actually vanish, and then in part II we will enter into the main question "how and why do the subnormal impulses vanish?"

Determination of the point at which subnormal impulses vanish. 1. According to Lucas and Adrian the Wedensky effect is produced because a series of subnormal impulses disappears during passage along narcotized area, becoming gradually smaller by decrement. If it is actually the case, then the Wedensky effect should appear earlier in a longer region of narcotized nerve than in a shorter one, i.e., in a longer stretch of narcotized nerve a less deep stage of narcosis is needed to abolish a series of subnormal impulses. To test this point two nerve-muscle preparations taken from



Figs. 1–3

the same Japanese toad were passed through a same narcotizing chamber as shown in figure 1 so that a different length of the two nerves could be exposed to the action of narcotic. The contraction of the attached muscle could be recorded on a slowly rotating kymograph. As narcosis proceeds tetanic stimuli (200 per second) were applied at the electrodes *A* and *B*, and the forms of the contraction curves were examined each time. As is well known, tetanic stimuli produce a continued tetanus in the beginning of narcosis, and at certain stage of narcosis the so-called initial twitch (Anfangszuckung) makes its appearance. However this initial twitch[1] is usually higher than a single twitch, suggesting that not only the first normal impulse but also some of the succeeding subnormal impulses are still reaching the muscle. Then the stage is at last reached at which the tetanic stimuli produce a contraction curve which is perfectly identical in

[1] This point will be dealt with in later pages (p. 478).

height and form with that produced by a single stimulus. It was shown by examination of the action current, which will be described later, that at this stage only the first (normal) impulse is reaching the muscle. Let us call, therefore, this effect "complete inhibition" hereafter. Thus it is easy to determine the time from the beginning of narcosis till appearance of complete inhibition by comparing the contraction curve with that produced by a single stimulus at the moment. The results are shown in tables 1 and 2.

Similar experiments were made with N. abdominalis (unbranched nerve) and similar results were obtained. It will be seen from these results that the complete inhibition appears, within experimental error, at the same time in both long and short regions of narcotized nerves, indicating that

TABLE 1

1.5 per cent cocaine-Ringer solution as a narcotic

NUMBER	TIME TILL COMPLETE INHIBITION APPEARS	
	Preparation X (15 mm.)	Preparation Y (30 mm.)
	minutes	*minutes*
1	67	65
2	47	47
3	40	42
4	74	77
5	75	75
6	36	37
7	39	39
8	65	64
9	51	50
10	73	73
11	68	68

TABLE 2

6.0 per cent alcohol-Ringer solution as a narcotic

NUMBER	TIME TILL COMPLETE INHIBITION APPEARS	
	Preparation X (15 mm.)	Preparation Y (30 mm.)
	minutes	*minutes*
1	42	42
2	69	67
3	52	61
4	61	61
5	97	98
6	45	45
7	84	85
8	65	63
9	75	75

TABLE 3

2.0 per cent cocaine-Ringer solution as a narcotic

NUMBER	TIME TILL COMPLETE INHIBITION APPEARS	
	Preparation X (4 mm.)	Preparation Y (30 mm.)
	minutes	*minutes*
1	33	29
2	41	29
3	47	35
4	33	27
5	53	38
6	43	19

the disappearance of the subnormal impulses is not due to decrement such as was postulated by Lucas and Adrian.

If, however, the narcotized region of the one nerve (preparation X) is made sufficiently short (below "limit length"), then markedly longer time is required, in this short region, till the complete inhibition appears (table 3). The reason is very clear from what we have described elsewhere (10). Below the "limit length" the narcosis does not proceed so rapidly as in the longer region on account of outward diffusion of narcotic and inward diffusion of normal tissue fluid at the edge of the narcotizing chamber.

2. The results of the experiments above described show that the subnormal impulses vanish somewhere at the beginning of the narcotized area.

This point can be confirmed by the following experiments. A nerve-muscle preparation was passed through a narcotizing chamber as shown in figure 2. W, A and B represent stimulating electrodes and the contraction of the muscle could be registered on a kymograph as in the previous experiment. To produce complete inhibition tetanic stimuli of 200 per second were applied at W. As soon as the stage of narcosis was reached at which complete inhibition appeared, the threshold as well as the just maximal current strength for *single* induction shock were determined at the electrodes A and B, *before* and then *during* the application of the tetanizing stimuli at W. The result of experiment 2 is shown below as an example.

		Coil distance without stimulation at W	Coil distance during stimulation at W
		mm.	*mm.*
At A	Threshold	265	35
	just maximal	235	30
At B	Threshold	205	205
	just maximal	170	170

It will be seen that at A, the outside electrode, the strength of the stimulus had to be increased from 265 mm. to 35 mm. (or from 235 mm. to 30 mm.) if applied during the tetanization which is producing inhibition, whereas at B, the inside electrode, it remained unchanged. If, during complete inhibition, a series of subnormal impulses starting from W would traverse actually the point B without reaching the muscle, then the threshold as well as the just maximal current strength at B ought to suffer a variation on account of the refractory periods due to these impulses. This result will easily be understood if it be assumed that the subnormal impulses are extinguished somewhere (p in fig. 2) at the beginning of the narcotized area and that therefore the point B is free from the subnormal impulses. The reason why a very strong electric stimulus applied at A was able to affect the muscle, whereas the series of subnormal impulses starting from W failed to pass the narcotized region, is found in the phenomenon of current spread. Any strong electric stimulus which can set up an impulse at a point more distal than the point P by current spread is able to affect the muscle. On the current spread we have reported elsewhere (11) in detail.

3. The above mentioned effect due to the current spread can be avoided by using mechanical stimulus. The following experiments were made to confirm the experimental results above mentioned and at the same time to eliminate the effect due to the current spread. Two nerve-muscle preparations (sciatic or N. abdominalis) were set up in a same narcotizing chamber as shown in figure 3. The narcosis proceeds and the stage is

reached at which tetanic stimuli given at the electrodes W and W' produce complete inhibition. At this stage, during tetanization at W, the preparation X was sharply cut at A (outside the chamber). The preparation Y was cut at B' (inside the chamber) during tetanization at W'. The former never gave muscular response, whereas the latter always did. Twenty-four experiments showed no exception. From this result, too, it will be seen, on one hand, that in complete inhibition the subnormal impulses do not traverse the point B', and on the other hand, that the muscle contraction produced by the strong electric stimulus (30 mm. coil distance) applied at A in the previous experiment was due to the current spread, because the mechanical stimulus (cutting) which cannot spread beyond the point P (see fig. 2) was never able to provoke muscle contraction.

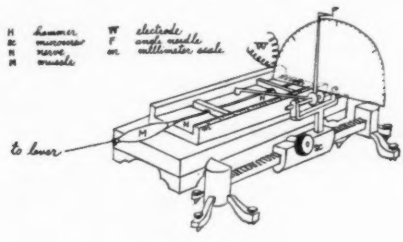
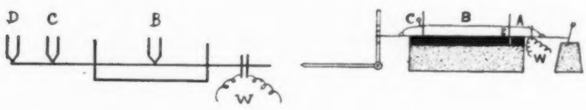


Fig. 4



Figs. 5 and 6

4. The series of the experiments described above demonstrates that the subnormal impulses are not extinguished by decrement, becoming gradually smaller as they pass along the narcotized region, but vanish at a certain upper point P between the middle point B and the upper chamber wall (see fig. 2). Let us, then, seek for the site of the point P at which they actually vanish. To meet this question we employed mechanical stimulation. The arrangement is reproduced in figure 4. A nerve-muscle preparation was set up in a shallow narcotizing chamber (30 mm. wide) and the slots through which the nerve was drawn were carefully plugged with vaseline. At the outside electrode W tetanic electric stimuli could be applied to detect the appearance of complete inhibition. The mechanical stimulus was given by means of a falling hammer made of aluminium. The smooth floor (ivory) of the narcotizing chamber served as an anvil. The corner of

the hammer-head was trimmed and carefully made smooth so that the nerve would not be injured. The hammer could be moved by a micrometer-screw along a millimeter-scale, so that the mechanical stimulus could be given at any desired point on the nerve. As a preliminary test, during tetanization producing complete inhibition, a fully maximal mechanical stimulus was applied to the nerve, first at a point just inside the upper chamber wall, and then at the middle of the chamber. The former gave no muscular response, whereas the latter did, as was expected from the previous experiment. This suggests that the point *P* in question at which the subnormal impulses actually vanish is situated between these two stimulated points. Therefore the second step was made in the following way.

TABLE 4

NUMBER	NARCOTIC	COMPLETE INHIBITION APPEARED AT	TIME TILL DETERMINATION AFTER COMPLETE INHIBITION APPEARED	DISTANCE BETWEEN P AND UPPER CHAMBER WALL
		minutes	*minutes*	*mm.*
1	0.5 per cent cocaine Ringer solution	50	0*	5
2		90	0	4
3		30	0	4
4		30	25	3
5		65	0	5
6		50	0	3
7		50	25	3
8		60	0	5
9	1.0 per cent cocaine Ringer solution	20	0	3
10		30	0	3
11		20	0	4
12		20	0	4
13		20	6	3
14		25	0	4

* 0 = determination was made as soon as complete inhibition appeared.

The mechanical stimulation was started from a point just inside the upper chamber wall downwards along the nerve towards the middle part of the chamber and thus the uppermost point was determined at which the mechanical stimulus just became effective to provoke muscle contraction. Then the mechanical stimulation was repeated in reversed direction starting from the middle part upwards and the lowest point was determined at which the mechanical stimulus just became ineffective. These two points thus determined nearly coincide in most of the experiments. Let us call this point "*P*" hereafter. The mean distances between *P* and the upper chamber wall are shown in table 4. The point *P* which is 3 to 5 mm. distal from the upper chamber wall indicates, although not very precisely, the

spot at which the series of the subnormal impulses coming from the outside electrode W vanish. The part of the narcotized nerve distal from this point, P, is free from impulses except the first (normal) one.

It is worth while to mention that this distance varies according to 1, the concentration of the narcotic used, and 2, the time elapsed between the appearance of complete inhibition and the determination. Table 5 shows the results obtained by changing the concentration of the narcotic. With

TABLE 5

NUMBER	NARCOTIC	COMPLETE INHIBITION APPEARED AT	TIME TILL DETERMINATION AFTER COMPLETE INHIBITION APPEARED	DISTANCE BETWEEN P AND UPPER CHAMBER WALL
		minutes	*minutes*	*mm.*
1	2.0 per cent	31	0	2
2	cocaine	16	0	3
3	Ringer	18	0	2
4	solution	25	0	3
5		18	0	3
6	0.3 per cent	145	0	5
7	Cocaine	94	0	5
8	Ringer	205	0	6
9	solution	100	0	6
10		145	0	5
11		130	0	6
2		100	0	5
13		135	0	5

TABLE 6

NUMBER	NARCOTIC	COMPLETE INHIBITION APPEARED AT	TIME TILL DETERMINATION AFTER COMPLETE INHIBITION APPEARED	DISTANCE BETWEEN P AND UPPER CHAMBER WALL
		minutes	*minutes*	*mm.*
1	0.3 per cent cocaine Ringer solution	143	0	5
			30	4
2		145	0	5
			30	4
3		130	0	6
			30	5
4		143	0	5
			30	4
5		138	0	5
			30	4
6		140	0	6
			30	5
7		A 115½	0	5
		B 117	30	4
8		A 122½	0	5
		B 128	30	4
9		A 90	0	6
		B 92	30	5
			60	4

2.0 per cent cocaine solution it is 2–3 mm., whereas it increases to 5–6 mm. in case of 0.3 per cent. In the experiments 1 to 6 in table 6 the determinations were made twice, first just after complete inhibition appeared and secondly 30 minutes later, in the same preparation. In order to avoid the error which might be caused by the mechanical stimuli previously applied, we used, in experiments 7 to 9, the two nerve-muscle preparations (A and B) taken from the same toad and narcotized them in the same chamber. For

example, in experiment 9 one preparation (A) showed complete inhibition at 90 minutes and the determination was immediately made. The distance was 6 mm. In the other preparation (B) which showed complete inhibition at 92 minutes the determination was made after 30 minutes. The distance was 5 mm. As this preparation (B) was still in good condition in this case, the determination was repeated once more after 60 minutes. It was decreased to 4 mm.

It must be noted that all these results are in complete agreement with what we have reported elsewhere (12) as effect of diffusion. As a matter of fact the uniform depth of narcosis is only reached at about 3–5 mm. inside of the chamber wall, within this distance the narcosis making a gradient on account of the diffusion. Therefore it is probable that when the stage of narcosis is only just reached at which complete inhibition is first produced, the series of subnormal impulses would be extinguished at the beginning (uppermost point) of the uniform narcosis, i.e., at a point about 3 to 5 mm. inside of the chamber wall. It was already reported (13) from this laboratory that the diffusion effect ("limit length") increases as the concentration of the narcotic is decreased.

From these observations and especially from those experiments to be described in part II it will be evident that for the interpretation of Wedensky effect the assumption of "transitional decrement" (14) is not necessary at all.

5. We have already mentioned (p. 472) that careful examination reveals two kinds of so-called initial twitch (Anfangszuckung) if carefully examined: the first one is somewhat higher than the single twitch produced by a single stimulus at the moment and as the narcosis deepens the stage is at last reached at which the second type appears, in which the contraction is perfectly identical with the single twitch in height and form. Let us now proceed to describe those experiments which were made to determine the number of the impulses passing through the narcotized region or reaching the muscle in these two kinds of initial twitch.

We employed the experimental arrangement previously described (see fig. 2, the electrodes *A* and *B* are not necessary in this case), and two lead-off electrodes were placed on the muscle, so that the action current (diphasic) and the height of the muscle contraction could be examined at the same time. The result of experiment 5 will be illustrated by an example (table 7). The initial twitch was first produced at 30 minutes of narcotization, but it was much higher than the single twitch produced by a single stimulus, and had three action currents. At 55 minutes the height of the initial twitch became equal with that of the single twitch. The number of action currents was reduced to only one at this moment and remained so through the succeeding stage of narcosis. In the previous description we named this effect in which the initial twitch shows the same height

with a single twitch as "complete inhibition". This result suggests that in complete inhibition only the first (normal) impulse is reaching the muscle.

The next experiments were conducted as follows: As soon as the stage of narcosis is reached at which complete inhibition appeared, the muscle was cut off and the monophasic action current was examined at two points on the nerve (see fig. 5), at *B*, the middle part of the narcotized nerve, and at *C*, a point outside of the distal chamber wall, the point *D* serving as an indifferent electrode. Twelve experiments were made and in every case we could find only one action current at *B* and also at *C*, although tetanic stimuli 200 per second were applied at the stimulating electrode *W*. It is very interesting to see that the photographic records of the action currents thus taken are completely indistinguishable from those obtained by applying a single induction shock at the electrode *W*. It indicates that in complete inhibition, as previously defined, the first (normal) impulse alone is passing through the narcotizing region, the subsequent subnormal

TABLE 7

14°C.

TIME OF NARCOSIS	HEIGHT OF CONTRACTION CURVE		NUMBER OF ACTION CURRENTS
	By tetanic stimuli	By single stimulus	
minutes	*mm.*	*mm.*	
30	32	18	3
45	30	16	2
55	12	12	1

impulses being extinguished at the beginning (*P* in fig. 2) of the narcotized area.

6. There remains an interesting observation of the same kind to be described which was made with a muscle. In this experiment there is an advantage that we can see with a magnifying glass, the point at which the series of the subnormal impulses actually vanishes.

A sartorius muscle taken from a large toad was passed through a narcotizing chamber as shown diagrammatically in figure 6, so that the muscle was divided into three parts A, B and C. The walls of the narcotizing chamber were made of rubber membrane stretched between the ebonite-frames. The small slot in the rubber membrane through which the muscle was drawn was so adjusted that the membrane pressed the muscle at the slot sufficiently to check the narcotizing solution from dripping, without preventing the propagated disturbance from passing through. Care was also taken so that the three parts of the muscle A, B and C had no mechanical influence on each other; in other words, the excitation evoked in the part A can certainly be conducted to the parts B and C, but the

shortening of the former can have no effect on the latter. This point can easily be confirmed by the following test: after narcotizing the part B to complete paralysis the stimulation at the electrode *W* caused the part A to contract, but the lever attached to the part C remained still. With this arrangement the tetanic stimuli (100 per second) applied at *W* produced a tetanic curve of the part C on the drum before narcotization. The narcosis (0.4 per cent cocaine-Ringer solution) was started, and at a certain stage of narcosis the contraction curve produced by the tetanic stimuli became equal to that produced by a single stimulus. This effect corresponds to the complete inhibition described in case of the nerve-muscle preparation. In this stage we can see with a magnifying glass that the muscle fibres in the part A show thickening or contraction according to the tetanic stimulation, but, on the contrary, those in the part B remain still except their uppermost region. We can trace the thickening (contraction) only to the point *P*, about 2 to 3 mm. from the upper chamber wall.

We proceeded further to register the "thickening curve" (Verdickungskurve) at three points *A*, *B* and *C*. In complete inhibition we obtained at *A*, of course, a curve of tetanic nature, whereas at *B* and *C* the tracings showed a single thickening.

The experiments were also made to test the action current instead of the "thickening curve". We will be content here with saying that the result sufficed to prove the same fact as the previous experiment.

It will be here noted that the "thickening" and also the electric response, which had once been reduced in the part B, grew larger on emerging into the part C. This point was in complete agreement with what we have (15) previously observed in the experiments on nerve conduction.

7. It will be noted that in this part I the subsequent impulses following the first normal one are said, for simplicity, to be subnormal. But it is not necessarily the case. In the late stage of narcosis complete inhibition can be produced by a series of impulses, each of which is normal in size. One example (expt. 2) will be mentioned briefly. In this case the normal nerve under the outside electrode *A* (see fig. 2) was recovered to the resting condition at 13.0σ after the first stimulus: at this moment the threshold returned to the normal value, and the electric response regained its normal size, too. At the outside electrode *A* tetanic stimuli of 40 per second were applied to see whether or not they could produce complete inhibition. At 300 minutes of narcosis (0.5 per cent cocaine-Ringer) they produced complete inhibition. Since the stimulus interval was 25.0σ, the subsequent impulses were all normal in size, and yet they were extinguished. This point can not be explained by decrement. The reason why the second (normal) impulse vanishes, whereas the first one can reach the muscle, was fully discussed elsewhere (16).

SUMMARY

1. Six kinds of experiments were made to determine the point in the narcotized nerve at which the series of subnormal impulses vanishes. All the experiments gave the same result, viz., that the subnormal impulses are extinguished, not by decrement as supposed by Lucas, but at the beginning of the narcotized area, about 3 to 5 mm. inside of the upper wall of the narcotizing chamber.

The authors wish to acknowledge that this research was made possible by the personal interest and generosity of Mr. G. Irie, Mr. I. Kadono, Mr. T. Maki and Mr. H. Yamaguchi. Our heartiest thanks are due to Dr. A. Forbes who has kindly improved the English.

The explanation of the third stage described by Wedensky (i.e., Hemmungsstadium) was completed, too. It will be published before long.

BIBLIOGRAPHY

(1) Wedensky: Pflüger's Arch., 1885, xxxvii, 69.
(2) Wedensky: Ibid., 1903, c, 1.
(3) Lucas: Journ. Physiol., 1911, xliii, 46.
(4) Adrian: Journ. Physiol., 1913, xlvi, 384. (See also recent paper by Beritoff: Zeitschr. f. Biol., 1923, lxxviii.)
(5) Kato: The theory of decrementless conduction in narcotized region of nerve. Nankodo, Tokyo, 1924. The further studies on decrementless conduction. Nankodo, Tokyo, 1926.
(6) Davis, Forbes, Brunswick and Hopkins: This Journal, 1926, lxxvi, 448.
(7) Davis and Rice: This Journal, 1928, lxxxv, 363.
(8) Kato and Teruuchi: Journ. Physiol., 1927, lxiv, 193.
(9) Kato et al: Keio Med. Sci., vii (Japanese); abst. in Physiol. Abst., 1927, xii, 351.
(10) Kato: The further studies on decrementless conduction. 1926, 49–52; see also Journ. Physiol., 1927, lxiv, 193.
(11) Kato: Ibid., 53.
(12) Kato: Ibid., 49. Also Kato and Teruuchi: Journ. Physiol., lxiv, 193.
(13) Kato and Teruuchi: Ibid., 198.
(14) Davis: Physiol. Reviews, 1926, vi, 555.
(15) Kato: The theory of decrementless conduction. 1924, 42.
(16) Kato: The further studies on decrementless conduction. 1926, 122.

The American Journal of Physiology is issued monthly by the American Physiological Society under the direction of the Council of the Society. From three to four volumes, each of about seven hundred and twenty pages are published yearly. The subscription price per volume in the United States and Canada is $7.50; in other countries, $8.00.

Manuscripts submitted for publication may be sent to any member of the Council or to the Managing Editor, Dr. D. R. Hooker, 19 W. Chase Street, Baltimore, Md. They should be **packed flat, carefully protected from injury, and insured.**

Each article should conclude with a **brief summary of results** suited to the needs of abstract journals.

References to literature should accord with the following arrangement. **In the text** author's name is to be followed by the year date of the publication in parenthesis, thus Smith (1911). If the author's name is not mentioned in the text, the parenthesis is to contain that name as well as the date, thus (Smith, 1911). If reference is made to more than one publication of an author in a given year, the year date is to be followed by the letters a, b, c, etc., thus Smith (1911a). **The bibliography** at the end of the article is to list all text references according to authors' names, with initials, alphabetically arranged, thus, Smith, J. C. 1911. Journ. Biol. Chem., xii, 306.

Authors are especially urged to verify personally the accuracy of their bibliographies by reference to the original sources, since inadvertent errors cause readers great inconvenience.

All figures for illustration must be submitted in such form as to admit of photographic reproduction without retouching or redrawing. Marginal letters or figures cannot be set in type and must, therefore, be drawn in India ink by the author and regarded as part of the figure. Unless specific instructions are given by authors, the printer will be allowed to use his judgment in determining the amount of reduction on all figures. The Journal cannot accept illustrations which, when reproduced for printing, exceed the size of a Journal page ($4\frac{1}{2} \times 7\frac{1}{2}$ inches), unless the author is prepared to pay the cost of an insert page.

Plotted curves must be drawn in India ink on **blue lined coördinate paper.** Blue ink will not show in reproduction, so that all guide lines, letters, etc., must be in India ink.

Best results are obtained when such figures are made proportional to the shape of the Journal page so that, when reduced, they will fit without undue loss of space.

(Contents continued)

ta-
of
is-

of
of
on
n

of
ein
'al-

of

lity
of

n
ical

tion
gen-

ase
gn.